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(54) Title: POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME			
(57) Abstract			
The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.			

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POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME

FIELD OF THE INVENTION

The present invention relates generally to the identification and isolation of novel DNA and to the recombinant production of novel polypeptides encoded by that DNA.

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BACKGROUND OF THE INVENTION

Extracellular proteins play an important role in the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

Secreted proteins have various industrial applications, including pharmaceuticals, diagnostics, biosensors and bioreactors. Most protein drugs available at present, such as thrombolytic agents, interferons, interleukins, erythropoietins, colony stimulating factors, and various other cytokines, are secretory proteins. Their receptors, which are membrane proteins, also have potential as therapeutic or diagnostic agents. Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., *Proc. Natl. Acad. Sci.*, 93:7108-7113 (1996); U.S. Patent No. 5,536,637].

Membrane-bound proteins and receptors can play an important role in the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. Such membrane-bound proteins and cell receptors include, but are not limited to, cytokine receptors, receptor kinases, receptor phosphatases, receptors involved in cell-cell interactions, and cellular adhesion molecules like selectins and integrins. For instance, transduction of signals that regulate cell growth and differentiation is regulated in part by phosphorylation of various cellular proteins. Protein tyrosine kinases, enzymes that catalyze that process, can also act as growth factor receptors. Examples include fibroblast growth factor receptor and nerve growth factor receptor.

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Membrane-bound proteins and receptor molecules have various industrial applications, including as pharmaceutical and diagnostic agents. Receptor immunoadhesins, for instance, can be employed as therapeutic agents to block receptor-ligand interaction. The membrane-bound proteins can also be employed for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. Efforts are being undertaken by both industry and academia to identify new, native receptor proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel receptor proteins.

We herein describe the identification and characterization of novel secreted and transmembrane polypeptides and novel nucleic acids encoding those polypeptides.

1. PRO241

Cartilage is a specialized connective tissue with a large extracellular matrix containing a dense network of collagen fibers and a high content of proteoglycan. While the majority of the proteoglycan in cartilage is aggrecan, which contains many chondroitin sulphate and keratin sulphate chains and forms multimolecular aggregates by binding with link protein to hyaluronan, cartilage also contains a number of smaller molecular weight proteoglycans. One of these smaller molecular weight proteoglycans is a protein called biglycan, a proteoglycan which is widely distributed in the extracellular matrix of various other connective tissues including tendon, sclera, skin, and the like. Biglycan is known to possess leucine-rich repeat sequences and two chondroitin sulphate/dermatan sulphate chains and functions to bind to the cell-binding domain of fibronectin so as to inhibit cellular attachment thereto. It is speculated that the small molecular weight proteoglycans such as biglycan may play important roles in the growth and/or repair of cartilage and in degenerative diseases such as arthritis. As such, there is an interest in identifying and characterizing novel polypeptides having homology to biglycan protein.

We herein describe the identification and characterization of novel polypeptides having homology to the biglycan protein, wherein those polypeptides are herein designated PRO241 polypeptides.

2. PRO243

Chordin (*Xenopus*, Xchd) is a soluble factor secreted by the Spemann organizer which has potent dorsalizing activity (Sasai *et al.*, *Cell* 79: 779-90 (1994); Sasai *et al.*, *Nature* 376: 333-36 (1995). Other dorsalizing factors secreted by the organizer are noggin (Smith and Harlan, *Cell* 70: 829-840 (1992); Lamb *et al.*, *Science* 262: 713-718 (1993) and follistatin (Hemmanti-Brivanlou *et al.*, *Cell* 77: 283-295 (1994). Chordin subdivides primitive ectoderm into neural versus non-neural domains, and induces notochord and muscle formation by the dorsalization of the mesoderm. It does this by functioning as an antagonist of the ventralizing BMP-4 signals. This inhibition is mediated by direct binding of chordin to BMP-4 in the extracellular space, thereby preventing BMP-4 receptor activation by BMP-4 (Piccolo *et al.*, *Develop. Biol.* 182: 5-20 (1996).

BMP-4 is expressed in a gradient from the ventral side of the embryo, while chordin is expressed in a gradient complementary to that of BMP-4. Chordin antagonizes BMP-4 to establish the low end of the BMP-4 gradient. Thus, the balance between the signal from chordin and other organizer-derived factors versus the BMP signal provides the ectodermal germ layer with its dorsal-ventral positional information. Chordin may also be involved in the dorsal-ventral patterning of the central nervous system (Sasai *et al.*, *Cell* 79: 779-90 (1994). It also induces exclusively anterior neural tissues (forebrain-type), thereby anteriorizing the neural type (Sasai *et al.*, *Cell* 79: 779-90 (1997). Given its role in neuronal induction and patterning, chordin may prove useful in the treatment

of neurodegenerative disorders and neural damage, e.g., due to trauma or after chemotherapy.

We herein describe the identification and characterization of novel polypeptides having homology to the chordin protein, wherein those polypeptides are herein designated PRO243 polypeptides.

3. PRO299

5 The notch proteins are involved in signaling during development. They may effect asymmetric development potential and may signal expression of other proteins involved in development. [See Robey, E., Curr. Opin. Genet. Dev., 7(4):551 (1997), Simpson, P., Curr. Opin. Genet. Dev., 7(4):537 (1997), Blobel, CP., Cell, 90(4):589 (1997)], Nakayama, H. et al., Dev. Genet., 21(1):21 (1997), Nakayama, H. et al., Dev. Genet., 21(1):21 (1997), Sullivan, S.A. et al., Dev. Genet., 20(3):208 (1997) and Hayashi, H. et al., Int. J. Dev. Biol., 40(6):1089(1996).]

10 Serrate-mediated activation of notch has been observed in the dorsal compartment of the Drosophila wing imaginal disc. Fleming et al., Development, 124(15):2973 (1997). Notch is of interest for both its role in development as well as its signaling abilities. Also of interest are novel polypeptides which may have a role in development and/or signaling.

We herein describe the identification and characterization of novel polypeptides having homology to the notch protein, wherein those polypeptides are herein designated PRO299 polypeptides.

4. PRO323

Dipeptidases are enzymatic proteins which function to cleave a large variety of different dipeptides and which are involved in an enormous number of very important biological processes in mammalian and non-mammalian organisms. Numerous different dipeptidase enzymes from a variety of different mammalian and non-mammalian organisms have been both identified and characterized. The mammalian dipeptidase enzymes play important roles in many different biological processes including, for example, protein digestion, activation, inactivation, or modulation of dipeptide hormone activity, and alteration of the physical properties of proteins and enzymes.

In light of the important physiological roles played by dipeptidase enzymes, efforts are being undertaken by both industry and academia to identify new, native dipeptidase homologs. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted and membrane-bound receptor proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., Proc. Natl. Acad. Sci., 93:7108-7113 (1996); U.S. Patent No. 5,536,637].

We herein describe the identification and characterization of novel polypeptides having homology to various dipeptidase enzymes, designated herein as PRO323 polypeptides.

5. PRO327

The anterior pituitary hormone prolactin is encoded by a member of the growth hormone/prolactin/placental lactogen gene family. In mammals, prolactin is primarily responsible for the development of the mammary gland and lactation. Prolactin functions to stimulate the expression of milk protein genes by increasing both gene transcription and mRNA half-life.

The physiological effects of the prolactin protein are mediated through the ability of prolactin to bind to a cell surface prolactin receptor. The prolactin receptor is found in a variety of different cell types, has a molecular mass of approximately 40,000 and is apparently not linked by disulfide bonds to itself or to other subunits. Prolactin

receptor levels are differentially regulated depending upon the tissue studied.

Given the important physiological roles played by cell surface receptor molecules *in vivo*, efforts are currently being undertaken by both industry and academia to identify new, native membrane-bound receptor proteins, including those which share sequence homology with the prolactin receptor. Many of these efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel membrane-bound receptor proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., *Proc. Natl. Acad. Sci.*, **93**:7108-7113 (1996); U.S. Patent No. 5,536,637].

We herein describe the identification and characterization of novel polypeptides having significant homology to the prolactin receptor protein, designated herein as PRO327 polypeptides.

6. PRO233

Studies have reported that the redox state of the cell is an important determinant of the fate of the cell. Furthermore, reactive oxygen species have been reported to be cytotoxic, causing inflammatory disease, including tissue necrosis, organ failure, atherosclerosis, infertility, birth defects, premature aging, mutations and malignancy. Thus, the control of oxidation and reduction is important for a number of reasons, including the control and prevention of strokes, heart attacks, oxidative stress and hypertension.

Oxygen free radicals and antioxidants appear to play an important role in the central nervous system after cerebral ischemia and reperfusion. Moreover, cardiac injury, related to ischaemia and reperfusion has been reported to be caused by the action of free radicals. In this regard, reductases, and particularly, oxidoreductases, are of interest. In addition, the transcription factors, NF-kappa B and AP-1, are known to be regulated by redox state and to affect the expression of a large variety of genes thought to be involved in the pathogenesis of AIDS, cancer, atherosclerosis and diabetic complications. Publications further describing this subject matter include Kelsey et al., *Br. J. Cancer*, **76**(7):852-854 (1997); Friedrich and Weiss, *J. Theor. Biol.*, **187**(4):529-540 (1997) and Pieulle et al., *J. Bacteriol.*, **179**(18):5684-5692 (1997). Given the physiological importance of redox reactions *in vivo*, efforts are currently being under taken to identify new, native proteins which are involved in redox reactions. We describe herein the identification and characterization of novel polypeptides which have homology to reductase, designated herein as PRO233 polypeptides.

7. PRO344

The complement proteins comprise a large group of serum proteins some of which act in an enzymatic cascade, producing effector molecules involved in inflammation. The complement proteins are of particular physiological importance in regulating movement and function of cells involved in inflammation. Given the physiological importance of inflammation and related mechanisms *in vivo*, efforts are currently being under taken to identify new, native proteins which are involved in inflammation. We describe herein the identification and characterization of novel polypeptides which have homology to complement proteins, wherein those polypeptides are herein designated as PRO344 polypeptides.

8. PRO347

Cysteine-rich proteins are generally proteins which have intricate three-dimensional structures and/or exist in multimeric forms due to the presence of numerous cysteine residues which are capable of forming disulfide

bridges. One well known cysteine-rich protein is the mannose receptor which is expressed in, among other tissues, liver where it serves to bind to mannose and transport it into liver cells. Other cysteine-rich proteins are known to play important roles in many other physiological and biochemical processes. As such, there is an interest in identifying novel cysteine-rich proteins. In this regard, Applicants describe herein the identification and characterization of novel cysteine-rich polypeptides that has significant sequence homology to the cysteine-rich secretory protein-3, designated herein as PRO347 polypeptides.

9. **PRO354**

Inter-alpha-trypsin inhibitor (ITI) is a large (Mr approximately 240,000) circulating protease inhibitor found in the plasma of many mammalian species. The intact inhibitor is a glycoprotein and consists of three glycosylated subunits that interact through a strong glycosaminoglycan linkage. The anti-trypsin activity of ITI is located on the smallest subunit (i.e., the light chain) of the complex, wherein that light chain is now known as the protein bikunin. The mature light chain consists of a 21-amino acid N-terminal sequence, glycosylated at Ser-10, followed by two tandem Kunitz-type domains, the first of which is glycosylated at Asn-45 and the second of which is capable of inhibiting trypsin, chymotrypsin and plasmin. The remaining two chains of the ITI complex are heavy chains which function to interact with the enzymatically active light chain of the complex.

Efforts are being undertaken by both industry and academia to identify new, native proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted and membrane-bound receptor proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., *Proc. Natl. Acad. Sci.*, 93:7108-7113 (1996); U.S. Patent No. 5,536,637]. We herein describe the identification and characterization of novel polypeptides having significant homology to the ITI heavy chain, designated in the present application as PRO354 polypeptides.

10. **PRO355**

Cytotoxic or regulatory T cell associated molecule or "CRTAM" protein is structurally related to the immunoglobulin superfamily. The CRTAM protein should be capable of mediating various immune responses. Antibodies typically bind to CRTAM proteins with high affinity. Zlotnik, A., *Faseb*, 10(6): A1037, Apr. 216, June 1996. Given the physiological importance of T cell antigens and immune processes *in vivo*, efforts are currently being under taken to identify new, native proteins which are involved in immune responses. See also Kennedy et al., U.S. Pat. No. 5,686,257 (1997). We describe herein the identification and characterization of novel polypeptides which have homology to CRTAM, designated in the present application as PRO355 polypeptides.

11. **PRO357**

Protein-protein interactions include receptor and antigen complexes and signaling mechanisms. As more is known about the structural and functional mechanisms underlying protein-protein interactions, protein-protein interactions can be more easily manipulated to regulate the particular result of the protein-protein interaction. Thus, the underlying mechanisms of protein-protein interactions are of interest to the scientific and medical community.

All proteins containing leucine-rich repeats are thought to be involved in protein-protein interactions. Leucine-rich repeats are short sequence motifs present in a number of proteins with diverse functions and cellular locations. The crystal structure of ribonuclease inhibitor protein has revealed that leucine-rich repeats correspond

to beta-alpha structural units. These units are arranged so that they form a parallel beta-sheet with one surface exposed to solvent, so that the protein acquires an unusual, nonglobular shape. These two features have been indicated as responsible for the protein-binding functions of proteins containing leucine-rich repeats. See, Kobe and Deisenhofer, Trends Biochem. Sci., 19(10):415-421 (Oct. 1994).

A study has been reported on leucine-rich proteoglycans which serve as tissue organizers, orienting and ordering collagen fibrils during ontogeny and are involved in pathological processes such as wound healing, tissue repair, and tumor stroma formation. Iozzo, R. V., Crit. Rev. Biochem. Mol. Biol., 32(2):141-174 (1997). Others studies implicating leucine rich proteins in wound healing and tissue repair are De La Salle, C., et al., Vouv. Rev. Fr. Hematol. (Germany), 37(4):215-222 (1995), reporting mutations in the leucine rich motif in a complex associated with the bleeding disorder Bernard-Soulier syndrome, Chlemetson, K. J., Thromb. Haemost. (Germany), 74(1):111-116 (July 1995), reporting that platelets have leucine rich repeats and Ruoslahti, E. I., et al., WO9110727-A by La Jolla Cancer Research Foundation reporting that decorin binding to transforming growth factor β has involvement in a treatment for cancer, wound healing and scarring. Related by function to this group of proteins is the insulin like growth factor (IGF), in that it is useful in wound-healing and associated therapies concerned with re-growth of tissue, such as connective tissue, skin and bone; in promoting body growth in humans and animals; and in stimulating other growth-related processes. The acid labile subunit (ALS) of IGF is also of interest in that it increases the half-life of IGF and is part of the IGF complex in vivo.

Another protein which has been reported to have leucine-rich repeats is the SLIT protein which has been reported to be useful in treating neuro-degenerative diseases such as Alzheimer's disease, nerve damage such as in Parkinson's disease, and for diagnosis of cancer, see, Artavanistsakonas, S. and Rothberg, J. M., WO9210518-A1 by Yale University. Also of interest is LIG-1, a membrane glycoprotein that is expressed specifically in glial cells in the mouse brain, and has leucine rich repeats and immunoglobulin-like domains. Suzuki, et al., J. Biol. Chem. (U.S.), 271(37):22522 (1996). Other studies reporting on the biological functions of proteins having leucine rich repeats include: Tayar, N., et al., Mol. Cell Endocrinol., (Ireland), 125(1-2):65-70 (Dec. 1996) (gonadotropin receptor involvement); Miura, Y., et al., Nippon Rinsho (Japan), 54(7):1784-1789 (July 1996) (apoptosis involvement); Harris, P. C., et al., J. Am. Soc. Nephrol., 6(4):1125-1133 (Oct. 1995) (kidney disease involvement).

Efforts are therefore being undertaken by both industry and academia to identify new proteins having leucine rich repeats to better understand protein-protein interactions. Of particular interest are those proteins having leucine rich repeats and homology to known proteins having leucine rich repeats such as the acid labile subunit of insulin-like growth factor. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted and membrane-bound proteins having leucine rich repeats. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., Proc. Natl. Acad. Sci., 93:7108-7113 (1996); U.S. Patent No. 5,536,637].

We describe herein the identification and characterization of novel polypeptides having homology to the acid labile subunit of insulin-like growth factor, designated in the present application as PRO357 polypeptides.

12. PRO715

Control of cell numbers in mammals is believed to be determined, in part, by a balance between cell proliferation and cell death. One form of cell death, sometimes referred to as necrotic cell death, is typically characterized as a pathologic form of cell death resulting from some trauma or cellular injury. In contrast, there is

another, "physiologic" form of cell death which usually proceeds in an orderly or controlled manner. This orderly or controlled form of cell death is often referred to as "apoptosis" [see, e.g., Barr et al., Bio/Technology, 12:487-493 (1994); Steller et al., Science, 267:1445-1449 (1995)]. Apoptotic cell death naturally occurs in many physiological processes, including embryonic development and clonal selection in the immune system [Itoh et al., Cell, 66:233-243 (1991)]. Decreased levels of apoptotic cell death have been associated with a variety of pathological conditions, including cancer, lupus, and herpes virus infection [Thompson, Science, 267:1456-1462 (1995)]. Increased levels of apoptotic cell death may be associated with a variety of other pathological conditions, including AIDS, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, retinitis pigmentosa, cerebellar degeneration, aplastic anemia, myocardial infarction, stroke, reperfusion injury, and toxin-induced liver disease [see, Thompson, supra].

Apoptotic cell death is typically accompanied by one or more characteristic morphological and biochemical changes in cells, such as condensation of cytoplasm, loss of plasma membrane microvilli, segmentation of the nucleus, degradation of chromosomal DNA or loss of mitochondrial function. A variety of extrinsic and intrinsic signals are believed to trigger or induce such morphological and biochemical cellular changes [Raff, Nature, 356:397-400 (1992); Steller, supra; Sachs et al., Blood, 82:15 (1993)]. For instance, they can be triggered by hormonal stimuli, such as glucocorticoid hormones for immature thymocytes, as well as withdrawal of certain growth factors [Watanabe-Fukunaga et al., Nature, 356:314-317 (1992)]. Also, some identified oncogenes such as *myc*, *rel*, and *E1A*, and tumor suppressors, like *p53*, have been reported to have a role in inducing apoptosis. Certain chemotherapy drugs and some forms of radiation have likewise been observed to have apoptosis-inducing activity [Thompson, supra].

Various molecules, such as tumor necrosis factor- α ("TNF- α "), tumor necrosis factor- β ("TNF- β " or "lymphotoxin- α "), lymphotoxin- β ("LT- β "), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), and Apo-2 ligand (also referred to as TRAIL) have been identified as members of the tumor necrosis factor ("TNF") family of cytokines [See, e.g., Gruss and Dower, Blood, 85:3378-3404 (1995); Pitti et al., J. Biol. Chem., 271:12687-12690 (1996); Wiley et al., Immunity, 3:673-682 (1995); Browning et al., Cell, 72:847-856 (1993); Armitage et al. Nature, 357:80-82 (1992)]. Among these molecules, TNF- α , TNF- β , CD30 ligand, 4-1BB ligand, Apo-1 ligand, and Apo-2 ligand (TRAIL) have been reported to be involved in apoptotic cell death. Both TNF- α and TNF- β have been reported to induce apoptotic death in susceptible tumor cells [Schmid et al., Proc. Natl. Acad. Sci., 83:1881 (1986); Dealtry et al., Eur. J. Immunol., 17:689 (1987)]. Zheng et al. have reported that TNF- α is involved in post-stimulation apoptosis of CD8-positive T cells [Zheng et al., Nature, 377:348-351 (1995)]. Other investigators have reported that CD30 ligand may be involved in deletion of self-reactive T cells in the thymus [Amakawa et al., Cold Spring Harbor Laboratory Symposium on Programmed Cell Death, Abstr. No. 10, (1995)].

Mutations in the mouse Fas/Apo-1 receptor or ligand genes (called *lpr* and *gld*, respectively) have been associated with some autoimmune disorders, indicating that Apo-1 ligand may play a role in regulating the clonal deletion of self-reactive lymphocytes in the periphery [Krammer et al., Curr. Op. Immunol., 6:279-289 (1994); Nagata et al., Science, 267:1449-1456 (1995)]. Apo-1 ligand is also reported to induce post-stimulation apoptosis in CD4-positive T lymphocytes and in B lymphocytes, and may be involved in the elimination of activated lymphocytes when their function is no longer needed [Krammer et al., supra; Nagata et al., supra]. Agonist mouse monoclonal antibodies specifically binding to the Apo-1 receptor have been reported to exhibit cell killing activity

that is comparable to or similar to that of TNF- α [Yonehara et al., *J. Exp. Med.*, 169:1747-1756 (1989)].

Induction of various cellular responses mediated by such TNF family cytokines is believed to be initiated by their binding to specific cell receptors. Two distinct TNF receptors of approximately 55-kDa (TNFR1) and 75-kDa (TNFR2) have been identified [Hohman et al., *J. Biol. Chem.*, 264:14927-14934 (1989); Brockhaus et al., *Proc. Natl. Acad. Sci.*, 87:3127-3131 (1990); EP 417,563, published March 20, 1991] and human and mouse cDNAs corresponding to both receptor types have been isolated and characterized [Loetscher et al., *Cell*, 61:351 (1990); Schall et al., *Cell*, 61:361 (1990); Smith et al., *Science*, 248:1019-1023 (1990); Lewis et al., *Proc. Natl. Acad. Sci.*, 88:2830-2834 (1991); Goodwin et al., *Mol. Cell. Biol.*, 11:3020-3026 (1991)]. The TNF family ligands identified to date, with the exception of lymphotoxin- α , are type II transmembrane proteins, whose C-terminus is extracellular. In contrast, most receptors in the TNF receptor (TNFR) family identified to date are type I transmembrane proteins. In both the TNF ligand and receptor families, however, homology identified between family members has been found mainly in the extracellular domain ("ECD"). Several of the TNF family cytokines, including TNF- α , Apo-1 ligand and CD40 ligand, are cleaved proteolytically at the cell surface; the resulting protein in each case typically forms a homotrimeric molecule that functions as a soluble cytokine. TNF receptor family proteins are also usually cleaved proteolytically to release soluble receptor ECDs that can function as inhibitors of the cognate cytokines.

Recently, other members of the TNFR family have been identified. Such newly identified members of the TNFR family include CAR1, HVEM and osteoprotegerin (OPG) [Brojatsch et al., *Cell*, 87:845-855 (1996); Montgomery et al., *Cell*, 87:427-436 (1996); Marsters et al., *J. Biol. Chem.*, 272:14029-14032 (1997); Simonet et al., *Cell*, 89:309-319 (1997)]. Unlike other known TNFR-like molecules, Simonet et al., *supra*, report that OPG contains no hydrophobic transmembrane-spanning sequence.

For a review of the TNF family of cytokines and their receptors, see Gruss and Dower, *supra*.

Applicants herein describe the identification and characterization of novel polypeptides having homology to members of the tumor necrosis factor family of polypeptides, designated herein as PRO715 polypeptides.

13. PRO353

The complement proteins comprise a large group of serum proteins some of which act in an enzymatic cascade, producing effector molecules involved in inflammation. The complement proteins are of particular importance in regulating movement and function of cells involved in inflammation. Given the physiological importance of inflammation and related mechanisms *in vivo*, efforts are currently being under taken to identify new, native proteins which are involved in inflammation. We describe herein the identification and characterization of novel polypeptides which have homology to complement proteins, designated herein as PRO353 polypeptides.

14. PRO361

The mucins comprise a family of glycoproteins which have been implicated in carcinogenesis. Mucin and mucin-like proteins are secreted by both normal and transformed cells. Both qualitative and quantitative changes in mucins have been implicated in various types of cancer. Given the medical importance of cancer, efforts are currently being under taken to identify new, native proteins which may be useful for the diagnosis or treatment of cancer.

The chitinase proteins comprise a family of which have been implicated in pathogenesis responses in plants. Chitinase proteins are produced by plants and microorganisms and may play a role in the defense of plants to injury. Given the importance of plant defense mechanisms, efforts are currently being under taken to identify new, native proteins which may be useful for modulation of pathogenesis-related responses in plants. We describe herein the identification and characterization of novel polypeptides which have homology to mucin and chitinase, designated in the present application as PRO361 polypeptides.

15. PRO365

Polypeptides such as human 2-19 protein may function as cytokines. Cytokines are low molecular weight proteins which function to stimulate or inhibit the differentiation, proliferation or function of immune cells. Cytokines often act as intercellular messengers and have multiple physiological effects. Given the physiological importance of immune mechanisms *in vivo*, efforts are currently being under taken to identify new, native proteins which are involved in effecting the immune system. We describe herein the identification and characterization of novel polypeptides which have homology to the human 2-19 protein, designated herein as PRO365 polypeptides.

SUMMARY OF THE INVENTION

1. PRO241

Applicants have identified a cDNA clone that encodes a novel polypeptide having homology to biglycan protein, wherein the polypeptide is designated in the present application as "PRO241".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO241 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO241 polypeptide having amino acid residues 1 to 379 of Figure 2 (SEQ ID NO:2), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides isolated PRO241 polypeptide. In particular, the invention provides isolated native sequence PRO241 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 379 of Figure 2 (SEQ ID NO:2). Another embodiment of the present invention is directed to a PRO241 polypeptide lacking the N-terminal signal peptide, wherein the PRO241 polypeptide comprises about amino acids 16 to 379 of the full-length PRO241 amino acid sequence (SEQ ID NO:2).

2. PRO243

Applicants have identified a cDNA clone (DNA35917-1207) that encodes a novel polypeptide, designated in the present application as "PRO243".

In one embodiment, the invention provides an isolated nucleic acid molecule having at least about 80% sequence identity to (a) a DNA molecule encoding a PRO243 polypeptide comprising the sequence of amino acids 24 to 954 of Fig. 4 (SEQ ID NO:7), or (b) the complement of the DNA molecule of (a). The sequence identity preferably is about 85%, more preferably about 90%, most preferably about 95%. In one aspect, the isolated nucleic acid has at least about 80%, preferably at least about 85%, more preferably at least about 90%, and most preferably at least about 95% sequence identity with a polypeptide having amino acid residues 1 to 954 of Fig. 4 (SEQ ID NO:7). Preferably, the highest degree of sequence identity occurs within the four (4) conserved cysteine clusters (amino acids 51 to 125; amino acids 705 to 761; amino acids 784 to 849; and amino acids 897 to 931) of Fig. 4 (SEQ

ID NO:7). In a further embodiment, the isolated nucleic acid molecule comprises DNA encoding a PRO243 polypeptide having amino acid residues 24 to 954 of Fig. 4 (SEQ ID NO:7), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. In another aspect, the invention provides a nucleic acid of the full length protein of clone DNA35917-1207, deposited with the ATCC under accession number ATCC 209508, alternatively the coding sequence of clone DNA35917-1207, deposited under accession number ATCC 209508.

In yet another embodiment, the invention provides isolated PRO243 polypeptide. In particular, the invention provides isolated native sequence PRO243 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 24 to 954 of Figure 4 (SEQ ID NO:7). Native PRO243 polypeptides with or without the native signal sequence (amino acids 1 to 23 in Figure 4 (SEQ ID NO:7), and with or without the initiating methionine are specifically included. Alternatively, the invention provides a PRO243 polypeptide encoded by the nucleic acid deposited under accession number ATCC 209508.

3. PRO299

Applicants have identified a cDNA clone that encodes a novel polypeptide, wherein the polypeptide is designated in the present application as "PRO299".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO299 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO299 polypeptide having amino acid residues 1 to 737 of Figure 9 (SEQ ID NO:15), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides isolated PRO299 polypeptide. In particular, the invention provides isolated native sequence PRO299 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 737 of Figure 9 (SEQ ID NO:15). An additional embodiment of the present invention is directed to an isolated extracellular domain of a PRO299 polypeptide.

4. PRO323

Applicants have identified a cDNA clone that encodes a novel polypeptide having homology to a microsomal dipeptidase protein, wherein the polypeptide is designated in the present application as "PRO323".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO323 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO323 polypeptide having amino acid residues 1 to 433 of Figure 13 (SEQ ID NO:24), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides isolated PRO323 polypeptide. In particular, the invention provides isolated native sequence PRO323 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 433 of Figure 13 (SEQ ID NO:24).

5. PRO327

Applicants have identified a cDNA clone that encodes a novel polypeptide having homology to prolactin receptor, wherein the polypeptide is designated in the present application as "PRO327".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO327 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO327 polypeptide having amino acid residues 1 to 422 of Figure 17 (SEQ ID NO:32), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

5 In another embodiment, the invention provides isolated PRO327 polypeptide. In particular, the invention provides isolated native sequence PRO327 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 422 of Figure 17 (SEQ ID NO:32).

6. PRO233

10 Applicants have identified a cDNA clone that encodes a novel polypeptide, wherein the polypeptide is designated in the present application as "PRO233".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO233 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO233 polypeptide having amino acid residues 1 to 300 of Figure 19 (SEQ ID NO:37), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides isolated PRO233 polypeptide. In particular, the invention provides isolated native sequence PRO233 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 300 of Figure 19 (SEQ ID NO:37).

20 7. PRO344

Applicants have identified a cDNA clone that encodes a novel polypeptide, wherein the polypeptides are designated in the present application as "PRO344".

25 In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO344 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO344 polypeptide having amino acid residues 1 to 243 of Figure 21 (SEQ ID NO:42), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

30 In another embodiment, the invention provides isolated PRO344 polypeptide. In particular, the invention provides isolated native sequence PRO344 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 243 of Figure 21 (SEQ ID NO:42).

8. PRO347

35 Applicants have identified a cDNA clone that encodes a novel polypeptide having homology to cysteine-rich secretory protein-3, wherein the polypeptide is designated in the present application as "PRO347".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO347 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO347 polypeptide having amino acid residues 1 to 455 of Figure 23 (SEQ ID NO:50), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency

conditions.

In another embodiment, the invention provides isolated PRO347 polypeptide. In particular, the invention provides isolated native sequence PRO347 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 455 of Figure 23 (SEQ ID NO:50).

5 **9. PRO354**

Applicants have identified a cDNA clone that encodes a novel polypeptide having homology to the heavy chain of the inter-alpha-trypsin inhibitor (ITI), wherein the polypeptide is designated in the present application as "PRO354".

10 In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO354 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO354 polypeptide having amino acid residues 1 to 694 of Figure 25 (SEQ ID NO:55), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

15 In another embodiment, the invention provides isolated PRO354 polypeptide. In particular, the invention provides isolated native sequence PRO354 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 694 of Figure 25 (SEQ ID NO:55).

20 **10. PRO355**

Applicants have identified a cDNA clone that encodes a novel polypeptide, wherein the polypeptide is designated in the present application as "PRO355".

25 In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO355 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO355 polypeptide having amino acid residues 1 to 440 of Figure 27 (SEQ ID NO:61), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

30 In another embodiment, the invention provides isolated PRO355 polypeptide. In particular, the invention provides isolated native sequence PRO355 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 440 of Figure 27 (SEQ ID NO:61). An additional embodiment of the present invention is directed to an isolated extracellular domain of a PRO355 polypeptide.

35 **11. PRO357**

Applicants have identified a cDNA clone that encodes a novel polypeptide having homology to insulin-like growth factor (IGF) acid labile subunit (ALS), wherein the polypeptide is designated in the present application as "PRO357".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO357 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO357 polypeptide having amino acid residues 1 through 598 of Figure 29 (SEQ ID NO:69), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency

conditions.

In another embodiment, the invention provides isolated PRO357 polypeptide. In particular, the invention provides isolated native sequence PRO357 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 through 598 of Figure 29 (SEQ ID NO:69). An additional embodiment of the present invention is directed to an isolated extracellular domain of a PRO357 polypeptide.

12. PRO715

Applicants have identified cDNA clones that encode novel polypeptides having homology to tumor necrosis factor family polypeptides, wherein the polypeptides are designated in the present application as "PRO715".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO715 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO715 polypeptide having amino acid residues 1 to 250 of Figure 31 (SEQ ID NO:76), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides isolated PRO715 polypeptide. In particular, the invention provides isolated native sequence PRO715 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 250 of Figure 31 (SEQ ID NO:76). An additional embodiment of the present invention is directed to an isolated extracellular domain of a PRO715 polypeptide.

13. PRO353

Applicants have identified a cDNA clone that encodes a novel polypeptide, wherein the polypeptides are designated in the present application as "PRO353".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO353 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO353 polypeptide having amino acid residues 1 to 281 of Figure 35 (SEQ ID NO:86), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides an isolated PRO353 polypeptide. In particular, the invention provides isolated native sequence PRO353 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 281 of Figure 35 (SEQ ID NO:86).

14. PRO361

Applicants have identified a cDNA clone that encodes a novel polypeptide, wherein the polypeptide is designated in the present application as "PRO361".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO361 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO361 polypeptide having amino acid residues 1 to 431 of Figure 37 (SEQ ID NO:91), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. The isolated nucleic acid sequence may comprise the cDNA insert of the vector deposited on February 5, 1998 as ATCC 209621 which includes the nucleotide sequence encoding PRO361.

In another embodiment, the invention provides isolated PRO361 polypeptide. In particular, the invention provides isolated native sequence PRO361 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 431 of Figure 37 (SEQ ID NO:91). An additional embodiment of the present invention is directed to an isolated extracellular domain of a PRO361 polypeptide having amino acids 1-379 of the amino acids sequence shown in Figure 37 (SEQ ID NO:91). Optionally, the PRO361 polypeptide is obtained or is obtainable by
5 expressing the polypeptide encoded by the cDNA insert of the vector deposited on February 5, 1998 as ATCC 209621.

15. PRO365

Applicants have identified a cDNA clone that encodes a novel polypeptide, wherein the polypeptide is
10 designated in the present application as "PRO365".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO365 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO365 polypeptide having amino acid residues 1 to 235 of Figure 39 (SEQ ID NO:99), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency
15 conditions. In another aspect, the isolated nucleic acid comprises DNA encoding the PRO365 polypeptide having amino acid residues 21 to 235 of Figure 39 (SEQ ID NO:99), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides isolated PRO365 polypeptide. In particular, the invention provides isolated native sequence PRO365 polypeptide, which in one embodiment, includes an amino acid sequence
20 comprising residues 1 to 235 of Figure 39 (SEQ ID NO:99). An additional embodiment of the present invention is directed to an amino acid sequence comprising residues 21 to 235 of Figure 39 (SEQ ID NO:99).

16. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding
25 any of the above or below described polypeptides. A host cell comprising any such vector is also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the above or below described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the above or below
30 described polypeptides fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises any of the above or below described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody.

35 In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences, wherein those probes may be derived from any of the above or below described nucleotide sequences.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence (SEQ ID NO:1) of a native sequence PRO241 cDNA, wherein SEQ ID NO:1 is a clone designated herein as "UNQ215" and/or "DNA34392-1170".

Figure 2 shows the amino acid sequence (SEQ ID NO:2) derived from the coding sequence of SEQ ID NO:1 shown in Figure 1. Also presented in Figure 2 are the locations of a putative signal peptide, a potential leucine zipper region and a potential N-glycosylation site.

Figure 3 shows a nucleotide sequence (SEQ ID NO:6) of a native sequence PRO243 cDNA, wherein SEQ ID NO:6 is a clone designated herein as "UNQ217" and/or "DNA35917-1207".

Figure 4 shows the amino acid sequence (SEQ ID NO:7) derived from the coding sequence of SEQ ID NO:6 shown in Figure 3.

Figure 5 shows the organization of the genomic clones in the THPO region of human chromosome 3q27-q28.

Figures 6A-B show the expression of PRO243 in human adult and fetal tissues. Fig. 6A is a northern blot of human adult and fetal tissues hybridized to a human chordin cDNA (PRO243) probe. The lower panel shows an actin control. Fig. 6B is a diagram of the human chordin (PRO243) cDNA with the positions of the codons encoding the conserved cysteine blocks shown. The extent of the probe used is showed by the solid line.

Figure 7 shows PRO243 *in situ* hybridization of adult human tissues giving a positive signal in the cleavage line of the developing synovial joint forming between the femoral head and acetabulum.

Figure 8 shows a nucleotide sequence (SEQ ID NO:14) of a native sequence PRO299 cDNA, wherein SEQ ID NO:14 is a clone designated herein as "UNQ262" and/or "DNA39976-1215".

Figure 9 shows the amino acid sequence (SEQ ID NO:15) derived from the coding sequence of SEQ ID NO:14 shown in Figure 8.

Figure 10 shows a nucleotide sequence designated herein as DNA28847 (SEQ ID NO:18).

Figure 11 shows a nucleotide sequence designated herein as DNA35877 (SEQ ID NO:19).

Figure 12 shows a nucleotide sequence (SEQ ID NO:23) of a native sequence PRO323 cDNA, wherein SEQ ID NO:23 is a clone designated herein as "UNQ284" and/or "DNA35595-1228".

Figure 13 shows the amino acid sequence (SEQ ID NO:24) derived from the coding sequence of SEQ ID NO:23 shown in Figure 12.

Figure 14 shows a single-stranded nucleotide sequence (SEQ ID NO:29) containing the nucleotide sequence (nucleotides 79-1416) of a chimeric fusion protein between a PRO323-derived polypeptide and a portion of an IgG constant domain, wherein the chimeric fusion protein is designated herein as "PRO454". The single-stranded nucleotide sequence (SEQ ID NO:29) encoding the PRO323/IgG fusion protein (PRO454) is designated herein as "DNA35872".

Figure 15 shows the amino acid sequence (SEQ ID NO:30) derived from nucleotides 79-1416 of the nucleotide sequence shown in Figure 14. The junction in the PRO454 amino acid sequence between the PRO323-derived sequences and the IgG-derived sequences appears between amino acids 415-416 in the figure.

Figure 16 shows a nucleotide sequence (SEQ ID NO:31) of a native sequence PRO327 cDNA, wherein SEQ ID NO:31 is a clone designated herein as "UNQ327" and/or "DNA38113-1230".

Figure 17 shows the amino acid sequence (SEQ ID NO:32) derived from the coding sequence of SEQ ID NO:31 shown in Figure 16.

Figure 18 shows a nucleotide sequence (SEQ ID NO:36) of a native sequence PRO233 cDNA, wherein SEQ ID NO:36 is a clone designated herein as "UNQ207" and/or "DNA34436-1238".

Figure 19 shows the amino acid sequence (SEQ ID NO:37) derived from the coding sequence of SEQ ID NO:36 shown in Figure 18.

Figure 20 shows a nucleotide sequence (SEQ ID NO:41) of a native sequence PRO344 cDNA, wherein SEQ ID NO:41 is a clone designated herein as "UNQ303" and/or "DNA40592-1242".

Figure 21 shows the amino acid sequence (SEQ ID NO:42) derived from the coding sequence of SEQ ID NO:41 shown in Figure 20.

Figure 22 shows a nucleotide sequence (SEQ ID NO:49) of a native sequence PRO347 cDNA, wherein SEQ ID NO:49 is a clone designated herein as "UNQ306" and/or "DNA44176-1244".

Figure 23 shows the amino acid sequence (SEQ ID NO:50) derived from the coding sequence of SEQ ID NO:49 shown in Figure 22.

Figure 24 shows a nucleotide sequence (SEQ ID NO:54) of a native sequence PRO354 cDNA, wherein SEQ ID NO:54 is a clone designated herein as "UNQ311" and/or "DNA44192-1246".

Figure 25 shows the amino acid sequence (SEQ ID NO:55) derived from the coding sequence of SEQ ID NO:54 shown in Figure 24.

Figure 26 shows a nucleotide sequence (SEQ ID NO:60) of a native sequence PRO355 cDNA, wherein SEQ ID NO:60 is a clone designated herein as "UNQ312" and/or "DNA39518-1247".

Figure 27 shows the amino acid sequence (SEQ ID NO:61) derived from the coding sequence of SEQ ID NO:60 shown in Figure 26.

Figure 28 shows a nucleotide sequence (SEQ ID NO:68) of a native sequence PRO357 cDNA, wherein SEQ ID NO:68 is a clone designated herein as "UNQ314" and/or "DNA44804-1248".

Figure 29 shows the amino acid sequence (SEQ ID NO:69) derived from the coding sequence of SEQ ID NO:68 shown in Figure 28.

Figure 30 shows a nucleotide sequence (SEQ ID NO:75) of a native sequence PRO715 cDNA, wherein SEQ ID NO:75 is a clone designated herein as "UNQ383" and/or "DNA52722-1229".

Figure 31 shows the amino acid sequence (SEQ ID NO:76) derived from the coding sequence of SEQ ID NO:75 shown in Figure 30.

Figure 32 shows a comparison of the amino acid sequences of human tumor necrosis factor- α (TNFA_HUMAN) (SEQ ID NO:77) with the amino acid sequence (SEQ ID NO:76) derived from nucleotides 114-863 of DNA52722-1229. Identical amino acids are boxed.

Figure 33 shows a comparison of the amino acid sequence (SEQ ID NO:76) derived from nucleotides 114-863 of DNA52722-1229 with the amino acid sequences of a variety of members of the tumor necrosis family of proteins (SEQ ID NOS:78-84). Identical amino acids are boxed.

Figure 34 shows a nucleotide sequence (SEQ ID NO:85) of a native sequence PRO353 cDNA, wherein SEQ ID NO:85 is a clone designated herein as "UNQ310" and/or "DNA41234-1242".

Figure 35 shows the amino acid sequence (SEQ ID NO:86) derived from the coding sequence of SEQ ID NO:85 shown in Figure 34.

Figure 36 shows a nucleotide sequence (SEQ ID NO:90) of a native sequence PRO361 cDNA, wherein SEQ ID NO:90 is a clone designated herein as "UNQ316" and/or "DNA45410-1250".

Figure 37 shows the amino acid sequence (SEQ ID NO:91) derived from the coding sequence of SEQ ID NO:90 shown in Figure 36.

Figure 38 shows a nucleotide sequence (SEQ ID NO:98) of a native sequence PRO365 cDNA, wherein SEQ ID NO:98 is a clone designated herein as "UNQ320" and/or "DNA46777-1253".

Figure 39 shows the amino acid sequence (SEQ ID NO:99) derived from the coding sequence of SEQ ID NO:98 shown in Figure 38.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (i.e., PRO/number) refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence PRO polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific PRO polypeptide (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO241 polypeptide is a mature or full-length native sequence PRO241 polypeptide comprising amino acids 1 to 379 of Figure 2 (SEQ ID NO:2), the native sequence PRO243 is a mature or full-length native sequence polypeptide comprising amino acids 24 to 954 of Fig. 4 (SEQ ID NO:7), with or without the N-terminal signal sequence (residues 1 to about 23), and with or without the initiating methionine at position 1, the native sequence PRO299 polypeptide is a mature or full-length native sequence PRO299 polypeptide comprising amino acids 1 to 737 of Figure 9 (SEQ ID NO:15) or the native sequence PRO299 polypeptide is an extracellular domain of the full-length PRO299 protein, wherein the putative transmembrane domain of the full-length PRO299 protein is encoded by nucleotides beginning at nucleotide 2022 as shown in Figure 8, the native sequence PRO323 polypeptide is a mature or full-length native sequence PRO323 polypeptide comprising amino acids 1 to 433 of Figure 13 (SEQ ID NO:24), the native sequence PRO327 polypeptide is a mature or full-length native sequence PRO327 polypeptide comprising amino acids 1 to 422 of Figure 17 (SEQ ID NO:32), the native sequence PRO233 polypeptide is a mature or full-length native sequence PRO233 polypeptide comprising amino acids 1 to 300 of Figure 19 (SEQ ID NO:37), the native sequence PRO344 polypeptide is a mature or full-length native sequence PRO344 polypeptide comprising amino acids 1 to 243 of Figure 21 (SEQ ID NO:42), the native sequence PRO347 polypeptide is a mature or full-length native sequence PRO347 polypeptide comprising amino acids 1 to 455 of Figure 23 (SEQ ID NO:50), the native sequence PRO354 polypeptide is a mature or full-length native sequence PRO354 polypeptide comprising amino acids 1 to 694 of Figure 25 (SEQ ID NO:55), the native sequence PRO355 polypeptide is a mature or full-length native sequence PRO355 polypeptide comprising amino acids 1 to 440 of Figure 27 (SEQ ID NO:61) or the native sequence PRO355 polypeptide is an extracellular domain of the full-length PRO355 protein, wherein the putative transmembrane domain of the full-length

PRO355 protein is encoded by nucleotides beginning at nucleotide 1138 as shown in Figure 26, the native sequence PRO357 polypeptide is a mature or full-length native sequence PRO357 polypeptide comprising amino acids 1 through 598 of Figure 29 (SEQ ID NO:69) or the native sequence PRO357 polypeptide is an extracellular domain of the full-length PRO357 protein, wherein the putative transmembrane domain of the full-length PRO357 protein is encoded by nucleotides 1518-1572 of SEQ ID NO:68, or alternatively, 1491-1572 of SEQ ID NO:68, the native sequence PRO715 polypeptide is a mature or full-length native sequence PRO715 polypeptide comprising amino acids 1 to 250 of Figure 31 (SEQ ID NO:76), the native sequence PRO353 polypeptide is a mature or full-length native sequence PRO353 polypeptide comprising amino acids 1 to 281 of Figure 35 (SEQ ID NO:86) or the native sequence PRO353 polypeptide is an extracellular domain of the full-length PRO353 protein, the native sequence PRO361 polypeptide is a mature or full-length native sequence PRO361 polypeptide comprising amino acids 1 to 431 of Figure 37 (SEQ ID NO:91) or the native sequence PRO361 polypeptide is an extracellular domain of the full-length PRO361 protein, wherein the putative transmembrane domain of the full-length PRO361 protein is encoded by nucleotides beginning at nucleotide 1363 as shown in Figure 36 and the native sequence PRO365 polypeptide is a mature or full-length native sequence PRO365 polypeptide comprising amino acids 1 to 235 of Figure 39 (SEQ ID NO:99).

The PRO polypeptide "extracellular domain" or "ECD" refers to a form of the PRO polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domains identified for the PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at least about 80% amino acid sequence identity with the full-length native sequence PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, and even more preferably at least about 90% amino acid sequence identity, yet more preferably at least about 95% amino acid sequence identity and most preferably at least about 99% amino acid sequence identity with the amino acid sequence of the full-length native amino acid sequence as disclosed herein.

With regard to PRO243 variants, the phrase "PRO243 variant" means an active PRO243 as defined below having at least about 80% amino acid sequence identity to (a) a DNA molecule encoding a PRO243 polypeptide, with or without its native signal sequence, or (b) the complement of the DNA molecule of (a). In a particular embodiment, the PRO243 variant has at least about 80% amino acid sequence homology with the PRO243 having the deduced amino acid sequence shown in Fig. 4 (SEQ ID NO:7) for a full-length native sequence PRO243. Such PRO243 variants include, for instance, PRO243 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the sequence of Fig. 4 (SEQ ID NO:7). Preferably, the nucleic acid or amino acid sequence identity is at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid

residues in the specific PRO polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. The preferred software alignment program is BLAST. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the PRO polypeptide nucleic acid. An isolated PRO polypeptide nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated PRO polypeptide nucleic acid molecules therefore are distinguished from the specific PRO polypeptide nucleic acid molecule as it exists in natural cells. However, an isolated PRO polypeptide nucleic acid molecule includes PRO polypeptide nucleic acid molecules contained in cells that ordinarily express the PRO polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is

operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

5 The term "antibody" is used in the broadest sense and specifically covers single anti-PRO polypeptide monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies) and anti-PRO polypeptide antibody compositions with polyepitopic specificity. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

10 "Active" or "activity" for the purposes herein refers to form(s) of PRO polypeptide which retain the biologic and/or immunologic activities of the specific native or naturally-occurring PRO polypeptide. As per PRO243, a preferred activity is the ability to bind to and affect, *e.g.*, block or otherwise modulate, an activity of chordin, wherein the activity preferably involves the regulation of notochord and muscle formation.

"Treatment" or "treating" refers to both therapeutic treatment and prophylactic or preventative measures.

15 Those in need of treatment include those already with the disorder as well as those prone to have the disorder of those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as sheep, dogs, horses, cats, cows, and the like. Preferably, the mammal herein is a human.

20 "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

25 The term "agonist" is used to refer to peptide and non-peptide analogs of the native PRO polypeptides (where native PRO polypeptide refers to pro-PRO polypeptide, pre-PRO polypeptide, prepro-PRO polypeptide, or mature PRO polypeptide) of the present invention and to antibodies specifically binding such native PRO polypeptides, provided that they retain at least one biological activity of a native PRO polypeptide. Preferably, the agonists of the present invention retain the qualitative binding recognition properties and receptor activation properties of the native PRO polypeptide.

30 The term "antagonist" is used to refer to a molecule inhibiting a biological activity of a native PRO polypeptide of the present invention wherein native PRO polypeptide refers to pro-PRO polypeptide, pre-PRO polypeptide, prepro-PRO polypeptide, or mature PRO polypeptide. Preferably, the antagonists herein inhibit the binding of a native PRO polypeptide of the present invention to a binding partner. A PRO polypeptide "antagonist" is a molecule which prevents, or interferes with, a PRO antagonist effector function (*e.g.* a molecule which prevents

or interferes with binding and/or activation of a PRO polypeptide receptor by PRO polypeptide). Such molecules can be screened for their ability to competitively inhibit PRO polypeptide receptor activation by monitoring binding of native PRO polypeptide in the presence and absence of the test antagonist molecule, for example. An antagonist of the invention also encompasses an antisense polynucleotide against the PRO polypeptide gene, which antisense polynucleotide blocks transcription or translation of the PRO polypeptide gene, thereby inhibiting its expression and biological activity.

"Stringent conditions" means (1) employing low ionic strength and high temperature for washing, for example, 0.015 sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C, or (2) employing during hybridization a denaturing agent, such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6/8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS. Yet another example is hybridization using a buffer of 10% dextran sulfate, 2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" are described in Sambrook *et al.*, *supra*, and include the use of a washing solution and hybridization conditions (*e.g.*, temperature, ionic strength, and %SDS) less stringent than described above. An example of moderately stringent conditions is a condition such as overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, *etc.*, as necessary to accommodate factors such as probe length and the like.

"Southern analysis" or "Southern blotting" is a method by which the presence of DNA sequences in a restriction endonuclease digest of DNA or a DNA-containing composition is confirmed by hybridization to a known, labeled oligonucleotide or DNA fragment. Southern analysis typically involves electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane support for analysis with a radiolabeled, biotinylated, or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989).

"Northern analysis" or "Northern blotting" is a method used to identify RNA sequences that hybridize to a known probe such as an oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ³²P, or by biotinylation, or with an enzyme. The RNA to be analyzed is usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook *et al.*, *supra*.

II. Compositions and Methods of the Invention

1. Full-length PRO241 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO241. In particular, Applicants have identified and isolated cDNA encoding a PRO241 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that portions of the PRO241 polypeptide have significant homology with the various biglycan proteins. Accordingly, it is presently believed that PRO241 polypeptide disclosed in the present application is a newly identified biglycan homolog polypeptide and may possess activity typical of biglycan proteins.

2. Full-length PRO243 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO243. In particular, Applicants have identified and isolated cDNA encoding a PRO243 polypeptide, as disclosed in further detail in the Examples below. Using BLAST, BLAST-2 and FastA sequence alignment computer programs, Applicants found that a full-length native sequence PRO243 (shown in Figure 4 and SEQ ID NO:7) has 50% amino acid sequence identity with African clawed frog and *Xenopus* chordin and 77% homology with rat chordin. Accordingly, it is presently believed that PRO243 disclosed in the present application is a newly identified member of the chordin protein family and may possess ability to influence notochord and muscle formation by the dorsalization of the mesoderm.

3. Full-length PRO299

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO299. In particular, Applicants have identified and isolated cDNA encoding a PRO299 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO299 polypeptide have significant homology with the notch protein. Accordingly, it is presently believed that PRO299 polypeptide disclosed in the present application is a newly identified member of the notch protein family and possesses signaling properties typical of the notch protein family.

4. Full-length PRO323 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO323. In particular, Applicants have identified and isolated cDNA encoding a PRO323 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO323 polypeptide have significant homology with various dipeptidase proteins. Accordingly, it is presently believed that PRO323 polypeptide disclosed in the present application is a newly identified dipeptidase homolog that has dipeptidase activity

5. Full-length PRO327 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO327. In particular, Applicants have identified and isolated cDNA encoding a PRO327 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that portions of the PRO327 polypeptide have significant
5 homology with various prolactin receptor proteins. Accordingly, it is presently believed that PRO327 polypeptide disclosed in the present application is a newly identified prolactin receptor homolog and has activity typical of a prolactin receptor protein.

6. Full-length PRO233 Polypeptides

10 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO233. In particular, Applicants have identified and isolated cDNA encoding a PRO233 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO233 polypeptide have significant homology with various reductase proteins. Applicants have also found that the DNA encoding the PRO233
15 polypeptide has significant homology with proteins from *Caenorhabditis elegans*. Accordingly, it is presently believed that PRO233 polypeptide disclosed in the present application is a newly identified member of the reductase family and possesses the ability to effect the redox state of a cell typical of the reductase family.

7. Full-length PRO344 Polypeptides

20 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO344. In particular, Applicants have identified and isolated cDNA encoding PRO344 polypeptides, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO344 polypeptide have significant homology with the human and mouse complement proteins. Accordingly, it is presently believed that the
25 PRO344 polypeptide disclosed in the present application is a newly identified member of the complement family and possesses the ability to affect the inflammation process as is typical of the complement family of proteins.

8. Full-length PRO347 Polypeptides

30 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO347. In particular, Applicants have identified and isolated cDNA encoding a PRO347 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that portions of the PRO347 polypeptide have significant homology with various cysteine-rich secretory proteins. Accordingly, it is presently believed that PRO347 polypeptide disclosed in the present application is a newly identified cysteine-rich secretory protein and may possess activity
35 typical of the cysteine-rich secretory protein family.

9. Full-length PRO354 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO354. In particular, Applicants have identified and isolated cDNA encoding a PRO354 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that portions of the PRO354 polypeptide have significant
5 homology with the inter-alpha-trypsin inhibitor heavy chain protein. Accordingly, it is presently believed that PRO354 polypeptide disclosed in the present application is a newly identified inter-alpha-trypsin inhibitor heavy chain homolog.

10. Full-length PRO355 Polypeptides

10 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO355. In particular, Applicants have identified and isolated cDNA encoding a PRO355 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO355 polypeptide have significant homology with the CRTAM protein. Applicants have also found that the DNA encoding the PRO355
15 polypeptide also has homology to the thymocyte activation and developmental protein, the H20A receptor, the H20B receptor, the poliovirus receptor and the *Cercopithecus aethiops* AGM delta 1 protein. Accordingly, it is presently believed that PRO355 polypeptide disclosed in the present application is a newly identified member of the CRTAM protein family.

20 11. Full-length PRO357 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO357. In particular, Applicants have identified and isolated cDNA encoding a PRO357 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO357 polypeptide have
25 significant homology with the acid labile subunit of insulin-like growth factor. Applicants have also found that non-coding regions of the DNA44804-1248 align with a human gene signature as described in WO 95/14772. Applicants have further found that non-coding regions of the DNA44804-1248 align with the adenovirus type 12/human recombinant viral DNA as described in Deuring and Doerfler, *Gene*, 26:283-289 (1983). Based on the coding region homology, it is presently believed that PRO357 polypeptide disclosed in the present application is a newly identified
30 member of the leucine rich repeat family of proteins, and particularly, is related to the acid labile subunit of insulin-like growth factor. As such, PRO357 is likely to be involved in binding mechanisms, and may be part of a complex.

12. Full-length PRO715 Polypeptides

35 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO715. In particular, Applicants have identified and isolated cDNA molecules encoding PRO715 polypeptides, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO715 polypeptides have significant homology with the various members of the tumor necrosis family of proteins. Accordingly, it is presently believed that the PRO715 polypeptides disclosed in the present application are newly identified members

of the tumor necrosis factor family of proteins.

13. Full-length PRO353 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO353. In particular, Applicants have identified and isolated cDNA encoding PRO353 polypeptides, as disclosed in further detail in the Examples below. Using BLAST and, FastA sequence alignment computer programs, Applicants found that various portions of the PRO353 polypeptides have significant homology with the human and mouse complement proteins. Accordingly, it is presently believed that the PRO353 polypeptides disclosed in the present application are newly identified members of the complement protein family and possesses the ability to effect the inflammation process as is typical of the complement family of proteins.

14. Full-length PRO361 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO361. In particular, Applicants have identified and isolated cDNA encoding a PRO361 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO361 polypeptide have significant homology with the mucin and chitinase proteins. Accordingly, it is presently believed that PRO361 polypeptide disclosed in the present application is a newly identified member of the mucin and/or chitinase protein families and may be associated with cancer, plant pathogenesis or receptor functions typical of the mucin and chitinase protein families, respectively.

15. Full-length PRO365 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO365. In particular, Applicants have identified and isolated cDNA encoding a PRO365 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the PRO365 polypeptide have significant homology with the human 2-19 protein. Accordingly, it is presently believed that PRO365 polypeptide disclosed in the present application is a newly identified member of the human 2-19 protein family.

16. PRO Polypeptide Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated that PRO polypeptide variants can be prepared. PRO polypeptide variants can be prepared by introducing appropriate nucleotide changes into the PRO polypeptide DNA, or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO polypeptides, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO polypeptides or in various domains of the PRO polypeptides described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO polypeptide that results in a change in the amino acid sequence of the PRO polypeptide as compared with the native sequence PRO polypeptide. Optionally

the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO polypeptide. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO polypeptide with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity in the *in vitro* assay described in the Examples below.

In particular embodiments, conservative substitutions of interest are shown in Table 1 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 1

	<u>Original Residue</u>	<u>Exemplary Substitutions</u>	<u>Preferred Substitutions</u>
20	Ala (A)	val; leu; ile	val
	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
25	Gln (Q)	asn	asn
	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe;	
30	Leu (L)	norleucine	leu
		norleucine; ile; val;	
		met; ala; phe	ile
	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
35	Phe (F)	leu; val; ile; ala; tyr	leu
	Pro (P)	ala	ala
	Ser (S)	thr	thr
	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr
40	Tyr (Y)	trp; phe; thr; ser	phe
	Val (V)	ile; leu; met; phe;	
		ala; norleucine	leu

Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- 5 (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)], cassette mutagenesis [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the desired PRO polypeptide variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

17. Modifications of PRO Polypeptides

Covalent modifications of PRO polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of the PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO polypeptide. Derivatization with bifunctional agents is useful, for instance, for crosslinking a PRO polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO polypeptide antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimide.

Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptides included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is

intended for purposes herein to mean deleting one or more carbohydrate moieties found in a native sequence PRO polypeptide, and/or adding one or more glycosylation sites that are not present in the native sequence PRO polypeptide, and/or alteration of the ratio and/or composition of the sugar residues attached to the glycosylation site(s).

Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO polypeptide (for O-linked glycosylation sites). The PRO polypeptide amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO polypeptide polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., Arch. Biochem. Biophys., 252:52 (1987) and by Edge et al., Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exoglycosidases as described by Thotakura et al., Meth. Enzymol., 138:350 (1987).

Another type of covalent modification of PRO polypeptides of the invention comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO polypeptides of the present invention may also be modified in a way to form a chimeric molecule comprising a PRO polypeptide fused to another, heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of the PRO polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO polypeptide. The presence of such epitope-tagged forms of the PRO polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of an IgG molecule.

Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide

[Martin et al., Science, 255:192-194 (1992)]; an α -tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

18. Preparation of PRO Polypeptides

5 The description below relates primarily to production of PRO polypeptides by culturing cells transformed or transfected with a vector containing the desired PRO polypeptide nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare the PRO polypeptide. For instance, the PRO polypeptide sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA
10 (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the desired PRO polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO polypeptide.

A. Isolation of DNA Encoding PRO Polypeptides

DNA encoding PRO polypeptides may be obtained from a cDNA library prepared from tissue believed to possess the desired PRO polypeptide mRNA and to express it at a detectable level. Accordingly, human PRO polypeptide DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described
20 in the Examples. The PRO polypeptide-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries can be screened with probes (such as antibodies to the desired PRO polypeptide or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described
25 in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding the desired PRO polypeptide is to use PCR methodology [Sambrook et al., *supra*; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences
30 selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ^{32}P -labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., *supra*.

35 Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined through sequence alignment using computer software programs such as BLAST, ALIGN, DNASTAR, and INHERIT which employ various algorithms to measure homology.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

5

B. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In
10 general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., supra.

Methods of transfection are known to the ordinarily skilled artisan, for example, CaPO_4 and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells.
15 The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects
20 of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown
25 et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110
30 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E.*
35 *coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325); and K5 772 (ATCC 53,635). These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which

has the complete genotype *tonA* ; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan^r*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan^r*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO polypeptide-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, Nature, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, Bio/Technology, 9: 968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al.*, J. Bacteriol., 737 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906; Van den Berg *et al.*, Bio/Technology, 8: 135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, J. Basic Microbiol., 28: 265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, Proc. Natl. Acad. Sci. USA, 76: 5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolytocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, Biochem. Biophys. Res. Commun., 112: 284-289 [1983]; Tilburn *et al.*, Gene, 26: 205-221 [1983]; Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, EMBO J., 4: 475-479 [1985]). Methylophilic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, The Biochemistry of Methylophilic Yeasts, 269 (1982).

Suitable host cells for the expression of glycosylated PRO polypeptides are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen. Virol., 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

C. Selection and Use of a Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding a desired PRO polypeptide may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an

appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO polypeptide of interest may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO polypeptide DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO polypeptide nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb et al., *Nature*, 282:39 (1979); Kingsman et al., *Gene*, 7:141 (1979); Tschemper et al., *Gene*, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO polypeptide nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose promoter systems [Chang et al., *Nature*, 275:615 (1978); Goeddel et al., *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the *tac* promoter [deBoer et al., *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the desired PRO polypeptide.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess et al., *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

5 Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytchrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

10 PRO polypeptide transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible
15 with the host cell systems.

Transcription of a DNA encoding the desired PRO polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an
20 enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO polypeptide coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated
25 cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO polypeptides.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO polypeptides in
30 recombinant vertebrate cell culture are described in Gething et al., *Nature*, 293:620-625 (1981); Mantei et al., *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

D. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional
35 Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that

upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to a PRO polypeptide DNA and encoding a specific antibody epitope.

E. Purification of Polypeptide

Forms of PRO polypeptides may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO polypeptides can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO polypeptides from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO polypeptide. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO polypeptide produced.

19. Uses for PRO Polypeptides

Nucleotide sequences (or their complement) encoding the PRO polypeptides of the present invention have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. PRO polypeptide-encoding nucleic acid will also be useful for the preparation of PRO polypeptides by the recombinant techniques described herein.

The full-length native sequence PRO polypeptide-encoding nucleic acid or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length PRO polypeptide gene or to isolate still other genes (for instance, those encoding naturally-occurring variants of the PRO polypeptide or PRO polypeptides from other species) which have a desired sequence identity to the PRO polypeptide nucleic acid sequences. Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from the nucleotide sequence of any of the DNA molecules disclosed herein or from genomic sequences including promoters, enhancer elements and introns of native sequence PRO polypeptide encoding DNA. By way of example, a screening method will comprise isolating the coding region of the PRO polypeptide gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as ³²P or ³⁵S, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the specific PRO polypeptide gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA

to determine which members of such libraries the probe hybridizes to. Hybridization techniques are described in further detail in the Examples below.

The ESTs disclosed in the present application may similarly be employed as probes, using the methods disclosed herein.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related PRO polypeptide sequences.

Nucleotide sequences encoding a PRO polypeptide can also be used to construct hybridization probes for mapping the gene which encodes that PRO polypeptide and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

When the coding sequence for the PRO polypeptide encodes a protein which binds to another protein, the PRO polypeptide can be used in assays to identify its ligands. Similarly, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Screening assays can be designed to find lead compounds that mimic the biological activity of a native PRO polypeptide or a ligand for the PRO polypeptide. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

Nucleic acids which encode a PRO polypeptide or its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding a PRO polypeptide of interest can be used to clone genomic DNA encoding the PRO polypeptide in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding the PRO polypeptide. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for PRO polypeptide transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding a PRO polypeptide introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding the PRO polypeptide. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of PRO polypeptides can be used to construct a PRO polypeptide "knock out" animal which has a defective or altered gene encoding the PRO polypeptide of interest as a result of

homologous recombination between the endogenous gene encoding the PRO polypeptide and altered genomic DNA encoding the PRO polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a PRO polypeptide can be used to clone genomic DNA encoding the PRO polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a PRO polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several
5 kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., *Cell*, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley,
10 in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability
15 to defend against certain pathological conditions and for their development of pathological conditions due to absence of the PRO polypeptide.

When *in vivo* administration of a PRO polypeptide is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 µg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery
20 is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

Where sustained-release administration of a PRO polypeptide is desired in a formulation with release
25 characteristics suitable for the treatment of any disease or disorder requiring administration of the PRO polypeptide, microencapsulation of the PRO polypeptide is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon- (rhIFN-), interleukin-2, and MN rgp120. Johnson *et al.*, *Nat. Med.*, 2: 795-799 (1996); Yasuda, *Biomed. Ther.*, 27: 1221-1223 (1993); Hora *et al.*, *Bio/Technology*, 8: 755-758 (1990); Cleland, "Design and Production of Single
30 Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, eds, (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins were developed using poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products
35 of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer," in: M. Chasin and R. Langer (Eds.), *Biodegradable Polymers as Drug Delivery Systems* (Marcel Dekker: New York, 1990), pp. 1-41.

For example, for a formulation that can provide a dosing of approximately 80 g/kg/day in mammals with a maximum body weight of 85 kg, the largest dosing would be approximately 6.8 mg of the PRO polypeptide per day. In order to achieve this dosing level, a sustained-release formulation which contains a maximum possible protein loading (15-20% w/w PRO polypeptide) with the lowest possible initial burst (<20%) is necessary. A continuous (zero-order) release of the PRO polypeptide from microparticles for 1-2 weeks is also desirable. In addition, the encapsulated protein to be released should maintain its integrity and stability over the desired release period.

PRO241 polypeptides of the present invention which possess biological activity related to that of the endogenous biglycan protein may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to employ the PRO241 polypeptides of the present invention for such purposes.

Chordin is a candidate gene for a dysmorphia syndrome known as Cornelia de Lange Syndrome (CDL) which is characterized by distinctive facial features (low anterior hairline, synophrys, antenerted nares, maxillary prognathism, long philtrum, 'carp' mouth), prenatal and postnatal growth retardation, mental retardation and, often but not always, upper limb abnormalities. There are also rare cases where CDL is present in association with thrombocytopenia. The gene for CDL has been mapped by linkage to 3q26.3 (OMIM #122470). Xchd involvement in early *Xenopus* patterning and nervous system development makes CHD an intriguing candidate gene. CHD maps to the appropriate region on chromosome 3. It is very close to THPO, and deletions encompassing both THPO and CHD could result in rare cases of thrombocytopenia and developmental abnormalities. *In situ* analysis of CD revealed that almost all adult tissues are negative for CHD expression, the only positive signal was observed in the cleavage line of the developing synovial joint forming between the femoral head and acetabulum (hip joint) implicating CHD in the development and presumably growth of long bones. Such a function, if disrupted, could result in growth retardation.

The human CHD amino acid sequence predicted from the cDNA is 50% identical (and 66% conserved) to Xchd. All 40 cysteines in the 4 cysteine-rich domains are conserved. These cysteine rich domains are similar to those observed in thrombospondin, procollagen and von Willebrand factor. Bornstein, P. *FASEB J* 6: 3290-3299 (1992); Hunt, L. & Barker, W. *Biochem. Biophys. Res. Commun.* 144: 876-882 (1987).

The human CHD locus (genomic PRO243) comprises 23 exons in 9.6 kb of genomic DNA. The initiating methionine is in exon 1 and the stop codon in exon 23. A CpG island is located at the 5' end of the gene, beginning approximately 100 bp 5' of exon 1 and extends through the first exon and ends within the first intron. The THPO and CHD loci are organized in a head-to-head fashion with approximately 2.2 kb separating their transcription start sites. At the protein level, PRO243 is 51% identical to *Xenopus* chordin (Xchd). All forty cysteines in the one amino terminal and three carboxy terminal cysteine-rich clusters are conserved.

PRO243 is a 954 amino acid polypeptide having a signal sequence at residues 1 to about 23. There are 4 cysteine clusters: (1) residues about 51 to about 125; (2) residues about 705 to about 761; (3) residues about 784 to about 849; and (4) residues about 897 to about 931. There are potential leucine zippers at residues about 315 to about 396, and N-glycosylation sites at residues 217, 351, 365 and 434.

PRO299 polypeptides and portions thereof which have homology to the notch protein may be useful for *in vivo* therapeutic purposes, as well as for various other applications. The identification of novel notch proteins and related molecules may be relevant to a number of human disorders such as those effecting development. Thus, the identification of new notch proteins and notch-like molecules is of special importance in that such proteins may serve as potential therapeutics for a variety of different human disorders. Such polypeptides may also play important roles

in biotechnological and medical research as well as various industrial applications. As a result, there is particular scientific and medical interest in new molecules, such as PRO299.

PRO323 polypeptides of the present invention which possess biological activity related to that of one or more endogenous dipeptidase proteins may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to employ the PRO323 polypeptides of the present invention for such purposes.

PRO327 polypeptides of the present invention which possess biological activity related to that of the endogenous prolactin receptor protein may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to employ the PRO327 polypeptides of the present invention for such purposes. PRO327 polypeptides which possess the ability to bind to prolactin may function both *in vitro* and *in vivo* as prolactin antagonists.

PRO233 polypeptides and portions thereof which have homology to reductase may also be useful for *in vivo* therapeutic purposes, as well as for various other applications. The identification of novel reductase proteins and related molecules may be relevant to a number of human disorders such as inflammatory disease, organ failure, atherosclerosis, cardiac injury, infertility, birth defects, premature aging, AIDS, cancer, diabetic complications and mutations in general. Given that oxygen free radicals and antioxidants appear to play important roles in a number of disease processes, the identification of new reductase proteins and reductase-like molecules is of special importance in that such proteins may serve as potential therapeutics for a variety of different human disorders. Such polypeptides may also play important roles in biotechnological and medical research, as well as various industrial applications. As a result, there is particular scientific and medical interest in new molecules, such as PRO233.

PRO344 polypeptides and portions thereof which have homology to complement proteins may also be useful for *in vivo* therapeutic purposes, as well as for various other applications. The identification of novel complement proteins and related molecules may be relevant to a number of human disorders such as effecting the inflammatory response of cells of the immune system. Thus, the identification of new complement proteins and complement-like molecules is of special importance in that such proteins may serve as potential therapeutics for a variety of different human disorders. Such polypeptides may also play important roles in biotechnological and medical research as well as various industrial applications. As a result, there is particular scientific and medical interest in new molecules, such as PRO344.

PRO347 polypeptides of the present invention which possess biological activity related to that of cysteine-rich secretory proteins may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to employ the PRO347 polypeptides of the present invention for such purposes.

PRO354 polypeptides of the present invention which possess biological activity related to that of the heavy chain of the inter-alpha-trypsin inhibitor protein may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to employ the PRO354 polypeptides of the present invention for such purposes.

PRO355 polypeptides and portions thereof which have homology to CRTAM may also be useful for *in vivo* therapeutic purposes, as well as for various other applications. The identification of novel molecules associated with T cells may be relevant to a number of human disorders such as conditions involving the immune system in general. Given that the CRTAM protein binds antibodies which play important roles in a number of disease processes, the identification of new CRTAM proteins and CRTAM-like molecules is of special importance in that such proteins may

serve as potential therapeutics for a variety of different human disorders. Such polypeptides may also play important roles in biotechnological and medical research, as well as various industrial applications. As a result, there is particular scientific and medical interest in new molecules, such as PRO355.

~~PRO357 can be used in competitive binding assays with ALS to determine its activity with respect to ALS.~~

Moreover, PRO357 can be used in assays to determine if it prolongs polypeptides which it may complex with to have longer half-lives *in vivo*. PRO357 can be used similarly in assays with carboxypeptidase, to which it also has homology. The results can be applied accordingly.

PRO715 polypeptides of the present invention which possess biological activity related to that of the tumor necrosis factor family of proteins may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to employ the PRO715 polypeptides of the present invention for such purposes. PRO715 polypeptides will be expected to bind to their specific receptors, thereby activating such receptors. Variants of the PRO715 polypeptides of the present invention may function as agonists or antagonists of their specific receptor activity.

PRO353 polypeptides and portions thereof which have homology to the complement protein may also be useful for *in vivo* therapeutic purposes, as well as for various other applications. The identification of novel complement proteins and related molecules may be relevant to a number of human disorders such as effecting the inflammatory response of cells of the immune system. Thus, the identification of new complement proteins complement-like molecules is of special importance in that such proteins may serve as potential therapeutics for a variety of different human disorders. Such polypeptides may also play important roles in biotechnological and medical research as well as various industrial applications. As a result, there is particular scientific and medical interest in new molecules, such as PRO353.

PRO361 polypeptides and portions thereof which have homology to mucin and/or chitinase proteins may also be useful for *in vivo* therapeutic purposes, as well as for various other applications. The identification of novel mucin and/or chitinase proteins and related molecules may be relevant to a number of human disorders such as cancer or those involving cell surface molecules or receptors. Thus, the identification of new mucin and/or chitinase proteins is of special importance in that such proteins may serve as potential therapeutics for a variety of different human disorders. Such polypeptides may also play important roles in biotechnological and medical research as well as various industrial applications. As a result, there is particular scientific and medical interest in new molecules, such as PRO361.

PRO365 polypeptides and portions thereof which have homology to the human 2-19 protein may also be useful for *in vivo* therapeutic purposes, as well as for various other applications. The identification of novel human 2-19 proteins and related molecules may be relevant to a number of human disorders such as modulating the binding or activity of cells of the immune system. Thus, the identification of new human 2-19 proteins and human 2-19 protein-like molecules is of special importance in that such proteins may serve as potential therapeutics for a variety of different human disorders. Such polypeptides may also play important roles in biotechnological and medical research as well as various industrial applications. As a result, there is particular scientific and medical interest in new molecules, such as PRO365.

20. Anti-PRO Polypeptide Antibodies

The present invention further provides anti-PRO polypeptide antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

A. Polyclonal Antibodies

5 The anti-PRO polypeptide antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent
10 to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

B. Monoclonal Antibodies

The anti-PRO polypeptide antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an
20 immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO polypeptide of interest or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with
25 an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells.
30 For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More
35 preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Rockville, Maryland. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the PRO polypeptide of interest. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined
5 by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.*, 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

10 The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and
15 sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the
20 recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-
25 combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are
30 deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

C. Humanized Antibodies

35 The anti-PRO polypeptide antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region

(CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones *et al.*, *Nature*, 321: 522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones *et al.*, *Nature*, 321: 522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991)).

D. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO polypeptide, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, *Nature*, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain

constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

5

E. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

15

21. Uses for Anti-PRO Polypeptide Antibodies

The anti-PRO polypeptide antibodies of the invention have various utilities. For example, anti-PRO polypeptide antibodies may be used in diagnostic assays for a PRO polypeptide, *e.g.*, detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ³H, ¹⁴C, ³²P, ³⁵S, or ¹²⁵I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter *et al.*, *Nature*, 144:945 (1962); David *et al.*, *Biochemistry*, 13:1014 (1974); Pain *et al.*, *J. Immunol. Meth.*, 40:219 (1981); and Nygren, *J. Histochem. and Cytochem.*, 30:407 (1982).

25

Anti-PRO polypeptide antibodies also are useful for the affinity purification of PRO polypeptide from recombinant cell culture or natural sources. In this process, the antibodies against the PRO polypeptide are immobilized on a suitable support, such as a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the PRO polypeptide to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the PRO polypeptide, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the PRO polypeptide from the antibody.

30

Chordin (CHD) is a candidate gene for a dysmorphia syndrome known as Cornelia de Lange Syndrome (CDL) which is characterized by distinctive facial features (low anterior hairline, synophrys, antenerted nares,

maxillary prognathism, long philtrum, 'carp' mouth), prenatal and postnatal growth retardation, mental retardation and, often but not always, upper limb abnormalities. There are also rare cases where CDL is present in association with thrombocytopenia. The gene for CDL has been mapped by linkage to 3q26.3 (OMIM #122470). Xchd (Xenopus chordin) involvement in early Xenopus patterning and nervous system development makes CHD an intriguing candidate gene. CHD maps to the appropriate region on chromosome 3. It is very close to THPO, and deletions encompassing both THPO and CHD could result in rare cases of thrombocytopenia and developmental abnormalities. In situ analysis of CD revealed that almost all adult tissues are negative for CHD expression, the only positive signal was observed in the cleavage line of the developing synovial joint forming between the femoral head and acetabulum (hip joint) implicating CHD in the development and presumably growth of long bones. Such a function, if disrupted, could result in growth retardation.

The human CHD amino acid sequence predicted from the cDNA is 50% identical (and 66% conserved) to Xchd. All 40 cysteines in the 4 cysteine-rich domains are conserved. These cysteine rich domains are similar to those observed in thrombospondin, procollagen and von Willebrand factor. Bornstein, P. *FASEB J* 6: 3290-3299 (1992); Hunt, L. & Barker, W. *Biochem. Biophys. Res. Commun.* 144: 876-882 (1987).

Antibodies to PRO243 chordin can be made which bind the polypeptide in conditions characterized by overexpression of PRO243.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Rockville, Maryland.

EXAMPLE 1: Extracellular Domain Homology Screening to Identify Novel Polypeptides and cDNA Encoding Therefor

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public databases (e.g., Dayhoff, GenBank), and proprietary databases (e.g. LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul and Gish, *Methods in Enzymology* 266: 460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons with a Blast score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, WA; (<http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>)).

Using this extracellular domain homology screen, consensus DNA sequences were assembled relative to the other identified EST sequences using phrap. In addition, the consensus DNA sequences obtained were often (but not always) extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible

using the sources of EST sequences discussed above.

Based upon the consensus sequences obtained as described above, oligonucleotides were then synthesized and used to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for a PRO polypeptide. Forward (-f) and reverse (-r) PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe (-p) sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

EXAMPLE 2: Isolation of cDNA clones by Amylase Screening

1. Preparation of oligo dT primed cDNA library

mRNA was isolated from a human tissue of interest using reagents and protocols from Invitrogen, San Diego, CA (Fast Track 2). This RNA was used to generate an oligo dT primed cDNA library in the vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). In this procedure, the double stranded cDNA was sized to greater than 1000 bp and the SalI/NotI linkered cDNA was cloned into XhoI/NotI cleaved vector. pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites.

2. Preparation of random primed cDNA library

A secondary cDNA library was generated in order to preferentially represent the 5' ends of the primary cDNA clones. Sp6 RNA was generated from the primary library (described above), and this RNA was used to generate a random primed cDNA library in the vector pSST-AMY.0 using reagents and protocols from Life Technologies (Super Script Plasmid System, referenced above). In this procedure the double stranded cDNA was sized to 500-1000 bp, linkered with blunt to NotI adaptors, cleaved with SfiI, and cloned into SfiI/NotI cleaved vector. pSST-AMY.0 is a cloning vector that has a yeast alcohol dehydrogenase promoter preceding the cDNA cloning sites and the mouse amylase sequence (the mature sequence without the secretion signal) followed by the yeast alcohol dehydrogenase terminator, after the cloning sites. Thus, cDNAs cloned into this vector that are fused in frame with amylase sequence will lead to the secretion of amylase from appropriately transfected yeast colonies.

3. Transformation and Detection

DNA from the library described in paragraph 2 above was chilled on ice to which was added electrocompetent DH10B bacteria (Life Technologies, 20 ml). The bacteria and vector mixture was then

electroporated as recommended by the manufacturer. Subsequently, SOC media (Life Technologies, 1 ml) was added and the mixture was incubated at 37°C for 30 minutes. The transformants were then plated onto 20 standard 150 mm LB plates containing ampicillin and incubated for 16 hours (37°C). Positive colonies were scraped off the plates and the DNA was isolated from the bacterial pellet using standard protocols, e.g. CsCl-gradient. The purified DNA was then carried on to the yeast protocols below.

5 The yeast methods were divided into three categories: (1) Transformation of yeast with the plasmid/cDNA combined vector; (2) Detection and isolation of yeast clones secreting amylase; and (3) PCR amplification of the insert directly from the yeast colony and purification of the DNA for sequencing and further analysis.

The yeast strain used was HD56-5A (ATCC-90785). This strain has the following genotype: MAT alpha, ura3-52, leu2-3, leu2-112, his3-11, his3-15, MAL⁺, SUC⁺, GAL⁺. Preferably, yeast mutants can be employed that 10 have deficient post-translational pathways. Such mutants may have translocation deficient alleles in *sec71*, *sec72*, *sec62*, with truncated *sec71* being most preferred. Alternatively, antagonists (including antisense nucleotides and/or ligands) which interfere with the normal operation of these genes, other proteins implicated in this post translation pathway (e.g., SEC61p, SEC72p, SEC62p, SEC63p, TDJ1p or SSA1p-4p) or the complex formation of these proteins may also be preferably employed in combination with the amylase-expressing yeast.

15 Transformation was performed based on the protocol outlined by Gietz et al., Nucl. Acid. Res., 20:1425 (1992). Transformed cells were then inoculated from agar into YEPD complex media broth (100 ml) and grown overnight at 30°C. The YEPD broth was prepared as described in Kaiser et al., Methods in Yeast Genetics, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 207 (1994). The overnight culture was then diluted to about 2 x 10⁶ cells/ml (approx. OD₆₀₀=0.1) into fresh YEPD broth (500 ml) and regrown to 1 x 10⁷ cells/ml (approx. 20 OD₆₀₀=0.4-0.5).

The cells were then harvested and prepared for transformation by transfer into GS3 rotor bottles in a Sorval GS3 rotor at 5,000 rpm for 5 minutes, the supernatant discarded, and then resuspended into sterile water, and centrifuged again in 50 ml falcon tubes at 3,500 rpm in a Beckman GS-6KR centrifuge. The supernatant was discarded and the cells were subsequently washed with LiAc/TE (10 ml, 10 mM Tris-HCl, 1 mM EDTA pH 7.5, 25 100 mM Li₂OOCCH₃), and resuspended into LiAc/TE (2.5 ml).

Transformation took place by mixing the prepared cells (100 µl) with freshly denatured single stranded salmon testes DNA (Lofstrand Labs, Gaithersburg, MD) and transforming DNA (1 µg, vol. < 10 µl) in microfuge tubes. The mixture was mixed briefly by vortexing, then 40% PEG/TE (600 µl, 40% polyethylene glycol-4000, 10 mM Tris-HCl, 1 mM EDTA, 100 mM Li₂OOCCH₃, pH 7.5) was added. This mixture was gently mixed and 30 incubated at 30°C while agitating for 30 minutes. The cells were then heat shocked at 42°C for 15 minutes, and the reaction vessel centrifuged in a microfuge at 12,000 rpm for 5-10 seconds, decanted and resuspended into TE (500 µl, 10 mM Tris-HCl, 1 mM EDTA pH 7.5) followed by recentrifugation. The cells were then diluted into TE (1 ml) and aliquots (200 µl) were spread onto the selective media previously prepared in 150 mm growth plates (VWR).

Alternatively, instead of multiple small reactions, the transformation was performed using a single, large 35 scale reaction, wherein reagent amounts were scaled up accordingly.

The selective media used was a synthetic complete dextrose agar lacking uracil (SCD-Ura) prepared as described in Kaiser et al., Methods in Yeast Genetics, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 208-210 (1994). Transformants were grown at 30°C for 2-3 days.

The detection of colonies secreting amylase was performed by including red starch in the selective growth media. Starch was coupled to the red dye (Reactive Red-120, Sigma) as per the procedure described by Biely et al., *Anal. Biochem.*, 172:176-179 (1988). The coupled starch was incorporated into the SCD-Ura agar plates at a final concentration of 0.15% (w/v), and was buffered with potassium phosphate to a pH of 7.0 (50-100 mM final concentration).

5 The positive colonies were picked and streaked across fresh selective media (onto 150 mm plates) in order to obtain well isolated and identifiable single colonies. Well isolated single colonies positive for amylase secretion were detected by direct incorporation of red starch into buffered SCD-Ura agar. Positive colonies were determined by their ability to break down starch resulting in a clear halo around the positive colony visualized directly.

10 4. Isolation of DNA by PCR Amplification

When a positive colony was isolated, a portion of it was picked by a toothpick and diluted into sterile water (30 μ l) in a 96 well plate. At this time, the positive colonies were either frozen and stored for subsequent analysis or immediately amplified. An aliquot of cells (5 μ l) was used as a template for the PCR reaction in a 25 μ l volume containing: 0.5 μ l KlenTaq (Clontech, Palo Alto, CA); 4.0 μ l 10 mM dNTP's (Perkin Elmer-Cetus); 2.5 μ l Kentaq buffer (Clontech); 0.25 μ l forward oligo 1; 0.25 μ l reverse oligo 2; 12.5 μ l distilled water. The sequence of the forward oligonucleotide 1 was:

5'-TGTAACGACGCGCCAGTTAAATAGACCTGCAATTATTAATCT-3' (SEQ ID NO:16)

The sequence of reverse oligonucleotide 2 was:

5'-CAGGAAACAGCTATGACCACCTGCACACCTGCAAATCCATT-3' (SEQ ID NO:17)

20 PCR was then performed as follows:

a.	Denature	92°C, 5 minutes
b.	3 cycles of:	
	Denature	92°C, 30 seconds
	Anneal	59°C, 30 seconds
25	Extend	72°C, 60 seconds
c.	3 cycles of:	
	Denature	92°C, 30 seconds
	Anneal	57°C, 30 seconds
	Extend	72°C, 60 seconds
30	d.	25 cycles of:
	Denature	92°C, 30 seconds
	Anneal	55°C, 30 seconds
	Extend	72°C, 60 seconds
35	e.	Hold
		4°C

The underlined regions of the oligonucleotides annealed to the ADH promoter region and the amylase region, respectively, and amplified a 307 bp region from vector pSST-AMY.0 when no insert was present. Typically, the first 18 nucleotides of the 5' end of these oligonucleotides contained annealing sites for the sequencing primers.

40 Thus, the total product of the PCR reaction from an empty vector was 343 bp. However, signal sequence-fused cDNA resulted in considerably longer nucleotide sequences.

Following the PCR, an aliquot of the reaction (5 μ l) was examined by agarose gel electrophoresis in a 1% agarose gel using a Tris-Borate-EDTA (TBE) buffering system as described by Sambrook et al., *supra*. Clones resulting in a single strong PCR product larger than 400 bp were further analyzed by DNA sequencing after

purification with a 96 Qiaquick PCR clean-up column (Qiagen Inc., Chatsworth, CA).

EXAMPLE 3: Isolation of cDNA Clones Encoding Human PRO241

A consensus DNA sequence was assembled relative to other EST sequences as described in Example 1 above. This consensus sequence is herein designated DNA30876. Based on the DNA30876 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO241.

PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-GGAAATGAGTGCAAACCCTC-3' (SEQ ID NO:3)

reverse PCR primer 5'-TCCCAAGCTGAACACTCATTCTGC-3' (SEQ ID NO:4)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30876 sequence which had the following nucleotide sequence

hybridization probe

5'-GGGTGACGGTGTTCATATCAGAATTGCAGAAGCAAACTGACCTCAGTT-3' (SEQ ID NO:5)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO241 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (LIB29).

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO241 [herein designated as UNQ215 (DNA34392-1170)] (SEQ ID NO:1) and the derived protein sequence for PRO241.

The entire nucleotide sequence of UNQ215 (DNA34392-1170) is shown in Figure 1 (SEQ ID NO:1). Clone UNQ215 (DNA34392-1170) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 234-236 and ending at the stop codon at nucleotide positions 1371-1373 (Figure 1). The predicted polypeptide precursor is 379 amino acids long (Figure 2). The full-length PRO241 protein shown in Figure 2 has an estimated molecular weight of about 43,302 daltons and a pI of about 7.30. Clone UNQ215 (DNA34392-1170) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209526.

Analysis of the amino acid sequence of the full-length PRO241 polypeptide suggests that it possess significant homology to the various biglycan proteoglycan proteins, thereby indicating that PRO241 is a novel biglycan homolog polypeptide.

EXAMPLE 4: Isolation of cDNA Clones Encoding Human PRO243 by Genomic Walking

Introduction: Human thrombopoietin (THPO) is a glycosylated hormone of 352 amino acids consisting of two domains. The N-terminal domain, sharing 50% similarity to erythropoietin, is responsible for the biological activity. The C-terminal region is required for secretion. The gene for thrombopoietin (THPO) maps to human chromosome 3q27-q28 where the six exons of this gene span 7 kilobase base pairs of genomic DNA (Gurney *et al.*, *Blood* 85: 981-988 (1995)). In order to determine whether there were any genes encoding THPO homologues located in close proximity to THPO, genomic DNA fragments from this region were identified and sequenced. Three P1 clones and one PAC clones (Genome Systems Inc., St. Louis, MO; cat. Nos. P1-2535 and PAC-6539) encompassing the THPO locus were isolated and a 140 kb region was sequenced using the ordered shotgun strategy (Chen *et al.*, *Genomics* 17: 651-656 (1993)), coupled with a PCR-based gap filling approach. Analysis reveals that the region is gene-rich

with four additional genes located very close to THPO: tumor necrosis factor-receptor type 1 associated protein 2 (TRAP2) and elongation initiation factor gamma (eIF4g), chloride channel 2 (CLCN2) and RNA polymerase II subunit hRPB17. While no THPO homolog was found in the region, four novel genes have been predicted by computer-assisted gene detection (GRAIL)(Xu *et al.*, *Gen. Engin.* **16**: 241-253 (1994), the presence of CpG islands (Cross, S. and Bird, A., *Curr. Opin. Genet. & Devel.* **5**: 109-314 (1995), and homology to known genes (as detected by WU-BLAST2.0)(Altschul and Gish, *Methods Enzymol.* **266**: 460-480 (1996) (<http://blast.wustl.edu/blast/README.html>).

P1 and PAC clones: The initial human P1 clone was isolated from a genomic P1 library (Genome Systems Inc., St. Louis, MO; cat. no.: P1-2535) screened with PCR primers designed from the THPO genomic sequence (A.L. Gurney, *et al.*, *Blood* **85**: 981-88 (1995). PCR primers were designed from the end sequences derived from this P1 clone were then used to screen P1 and PAC libraries (Genome Systems, Cat. Nos.: P1-2535 & PAC-6539) to identify overlapping clones.

Ordered Shotgun Strategy: The Ordered Shotgun Strategy (OSS) (Chen *et al.*, *Genomics* **17**: 651-656 (1993)) involves the mapping and sequencing of large genomic DNA clones with a hierarchical approach. The P1 or PAC clone was sonicated and the fragments subcloned into lambda vector (λ Bluestar) (Novagen, Inc., Madison, WI; cat. no. 69242-3). The lambda subclone inserts were isolated by long-range PCR (Barnes, W. *Proc. Natl. Acad. Sci. USA* **91**: 2216-2220 (1994) and the ends sequenced. The lambda-end sequences were overlapped to create a partial map of the original clone. Those lambda clones with overlapping end-sequences were identified, the insets subcloned into a plasmid vector (pUC9 or pUC18) and the ends of the plasmid subclones were sequenced and assembled to generate a contiguous sequence. This directed sequencing strategy minimizes the redundancy required while allowing one to scan for and concentrate on interesting regions.

In order to define better the THPO locus and to search for other genes related to the hematopoietin family, four genomic clones were isolated from this region by PCR screening of human P1 and PAC libraries (Genome System, Inc., Cat. Nos.: P1-2535 and PAC-6539). The sizes of the genomic fragments are as follows: P1.t is 40 kb; P1.g is 70 kb; P1.u is 70 kb; and PAC.z is 200 kb. The relationships between these four genomic clones are illustrated in Figure 5. Approximately 80% of the 200 kb genomic DNA region was sequenced by the Ordered Shotgun Strategy (OSS) (Chen *et al.*, *Genomics* **17**: 651-56 (1993), and assembled into contigs using AutoAssembler™ (Applied Biosystems, Perkin Elmer, Foster City, CA, cat. no. 903227). The preliminary order of these contigs was determined by manual analysis. There were 46 contigs and filling in the gaps was employed. Table 2 summarized the number and sizes of the gaps.

Table 2

Summary of the gaps in the 140 kb region

<u>Size of gap</u>	<u>number</u>
< 50 bp	13
50-150 bp	7
150-300 bp	7
300-1000 bp	10
1000-5000 bp	7
> 5000 bp	2 (15,000 bp)

- 10 *DNA sequencing:* ABI DYE-primer™ chemistry (PE Applied Biosystems, Foster City, CA; Cat. No.: 402112) was used to end-sequence the lambda and plasmid subclones. ABI DYE-terminator™ chemistry (PE Applied Biosystems, Foster City, CA, Cat. No: 403044) was used to sequence the PCR products with their respective PCR primers. The sequences were collected with an ABI377 instrument. For PCR products larger than 1kb, walking primers were used. The sequences of contigs generated by the OSS strategy in AutoAssembler™ a (PE Applied Biosystems, Foster City, CA; Cat. No: 903227) and the gap-filling sequencing trace files were imported into Sequencher™ (Gene Codes Corp., Ann Arbor, MI) for overlapping and editing.

- 20 *PCR-Based gap filling Strategy:* Primers were designed based on the 5'- and 3'-end sequenced of each contig, avoiding repetitive and low quality sequence regions. All primers were designed to be 19-24-mers with 50-70% G/C content. Oligos were synthesized and gel-purified by standard methods.

- 25 Since the orientation and order of the contigs were unknown, permutations of the primers were used in the amplification reactions. Two PCR kits were used: first, XL PCR kit (Perkin Elmer, Norwalk, CT; Cat. No.: N8080205), with extension times of approximately 10 minutes; and second, the Taq polymerase PCR kit (Qiagen Inc., Valencia, CA; Cat. No.: 201223) was used under high stringency conditions if smeared or multiple products were observed with the XL PCR kit. The main PCR product from each successful reactions was extracted from a 0.9% low melting agarose gel and purified with the GeneClean DNA Purification kit prior to sequencing.

- 30 *Analysis:* The identification and characterization of coding regions was carried out as follows: First, repetitive sequences were masked using RepeatMasker (A.F.A. Smit & P. Green, http://ftp.genome.washington.edu/RM/RM_details.html) which screens DNA sequences in FastA format against a library of repetitive elements and returns a masked query sequence. Repeats not masked were identified by comparing the sequence to the GenBank database using WUBLAST (Altschul, S & Gish, W., *Methods Enzymol.* 266: 460-480 (1996) and were masked manually.

- 35 Next, known genes were revealed by comparing the genomic regions against Genentech's protein database using the WUBLAST2.0 algorithm and then annotated by aligning the genomic and cDNA sequences for each gene, respectively, using a Needleman-Wunch (Needleman and Wunsch, *J. Mol. Biol.* 48: 443-453 (1970) algorithm to find regions of local identity between sequences which are otherwise largely dissimilar. The strategy results in detection of all exons of the five known genes in the region, THPO, TRAP2, eIF4g, CLCN2 and hRPB17 (Table 3).

Table 3

Summary of known genes located in the 140 kb region analyzed

<u>Known genes</u>	<u>Map position</u>
eukaryotic translation initiation factor 4-gamma	3q27-qter
thrombopoietin	3q26-q27
5 chloride channel 2	3q26-qter
TNF receptor associated protein 2	not previously mapped
RNA polymerase II subunit hRPB17	not previously mapped

Finally, novel transcription units were predicted using a number of approaches. CpG islands (S. Cross & Bird, A., *Curr. Opin. Genet. Dev.* 5: 109-314 (1995) islands were used to define promoter regions and were identified as clusters of sites cleaved by enzymes recognizing GC-rich, 6 or 8-mer palindromic sequences. CpG islands are usually associated with promoter regions of genes. WUBLAST2.0 analysis of short genomic regions (10-20 kb) versus GenBank revealed matches to ESTs. The individual EST sequences (or where possible, their sequence chromatogram files) were retrieved and assembled with Sequencher to provide a theoretical cDNA sequence (designated herein as DNA34415). GRAIL2 (ApoCom Inc., Knoxville, TN, command line version for the DEC alpha) was used to predict a novel exon. The five known genes in the region served as internal controls for the success of the GRAIL algorithm.

Isolation: Chordin cDNA clones were isolated from an oligo-dT-primed human fetal lung library. Human fetal lung polyA⁺ RNA was purchased from Clontech (cat #6528-1, lot #43777) and 5 mg used to construct a cDNA library in pKR5B (Genentech, LIB26). The 3'-primer (pGACTAGTTCTAGATCGCGAGCGGCCGCCCTTTTTTTTTTTTTT) (SEQ ID NO:8) and the 5'-linker (pCGGACGCGTGGGGCCTGCGACCCAGCT) (SEQ ID NO:9) were designed to introduce Sall and NotI restriction sites. Clones were screened with oligonucleotide probes designed from the putative human chordin cDNA sequence (DNA34415) deduced by manually "splicing" together the proposed genomic exons of the gene. PCR primers flanking the probes were used to confirm the identity of the cDNA clones prior to sequencing.

The screening oligonucleotides probes were the following:

OLI5640 34415.p1 5'-GCCGCTCCCCGAACGGGCAGCGGCTCCTTCTCAGAA-3' (SEQ ID NO:10) and
 OLI5642 34415.p2 5'-GGCGCACAGCACGCAGCGCATCACCCGAATGGCTC-3' (SEQ ID NO:11); and the
 30 flanking probes used were the following:
 OLI5639 34415.f1 5'-GTGCTGCCCATCCGTTCTGAGAAGGA-3' (SEQ ID NO:12) and
 OLI5643 34415.r 5'-GCAGGGTGCTCAAACAGGACAC-3' (SEQ ID NO:13).

EXAMPLE 5: Northern Blot and *in situ* RNA Hybridization Analysis of PRO243

35 Expression of PRO243 mRNA in human tissues was examined by Northern blot analysis. Human polyA⁺ RNA blots derived from human fetal and adult tissues (Clontech, Palo Alto, CA; Cat. Nos. 7760-1 and 7756-1) were hybridized to a ³²P-labelled cDNA fragments probe based on the full length PRO243 cDNA. Blots were incubated with the probes in hybridization buffer (5X SSPE; 2X Denhardt's solution; 100 mg/mL denatured sheared salmon sperm DNA; 50% formamide; 2% SDS) for 60 hours at 42°C. The blots were washed several times in 2X SSC;

0.05% SDS for 1 hour at room temperature, followed by a high stringency wash 30 minute wash in 0.1X SSC; 0.1% SDS at 50°C and autoradiographed. The blots were developed after overnight exposure by phosphorimager analysis (Fuji).

As shown in Fig. 6, PRO243 mRNA transcripts were detected. Analysis of the expression pattern showed the strongest signal of the expected 4.0 kb transcript in adult and fetal liver and a very faint signal in the adult kidney.

5 Fetal brain, lung and kidney were negative, as were adult heart, brain, lung and pancreas. Smaller transcripts were observed in placenta (2.0 kb), adult skeletal muscle (1.8 kb) and fetal liver (2.0 kb).

In situ hybridization of adult human tissue of PRO243 gave a positive signal in the cleavage line of the developing synovial joint forming between the femoral head and acetabulum. All other tissues were negative. Additional sections of human fetal face, head, limbs and mouse embryos were examined. Expression in human fetal
10 tissues was observed adjacent to developing limb and facial bones in the perosteal mesenchyme. The expression was highly specific and was often adjacent to areas undergoing vascularization. Expression was also observed in the developing temporal and occipital lobes of the fetal brain, but was not observed elsewhere in the brain. In addition, expression was seen in the ganglia of the developing inner ear. No expression was seen in any of the mouse tissues with the human probes (see Figure 7).

15 *In situ* hybridization was performed using an optimized protocol, using PCR-generating ³²P-labeled riboprobes. (Lu and Gillett, *Cell Vision* 1: 169-176 (1994)). Formalin-fixed, paraffin-embedded human fetal and adult tissues were sectioned, deparaffinized, deproteinized in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett (1994). A [³²P]-UTP-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak
20 NTB2 nuclear track emulsion and exposed for 4 weeks.

EXAMPLE 6: Isolation of cDNA clones Encoding Human PRO299

A cDNA sequence designated herein as DNA28847 (Figure 10; SEQ ID NO:18) was isolated as described in Example 2 above. After further analysis, a 3' truncated version of DNA28847 was found and is herein designated
25 DNA35877 (Figure 11; SEQ ID NO:19). Based on the DNA35877 sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO299. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus
30 sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

Forward and reverse PCR primers were synthesized:

35 forward PCR primer (35877.f1) 5'-CTCTGGAAGGTCACGGCCACAGG-3'
(SEQ ID NO:20)
reverse PCR primer (35877.r1) 5'-CTCAGTTCGGTTGGCAAAGCTCTC-3'
(SEQ ID NO:21)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the DNA35877 sequence which had the following nucleotide sequence

hybridization probe (35877.p1)

5'-CAGTGCTCCCTCATAGATGGACGAAAGTGTGACCCCCCTTTCAGGCGAGAGCTTTGCCAACCGAACTGA-3' (SEQ ID NO:22)

5 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO299 sequence using the probe oligonucleotide.

RNA for construction of the cDNA libraries was isolated from human fetal brain tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to Sall hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO299 [herein designated as UNQ262 (DNA39976-1215)] (SEQ ID NO:14) and the derived protein sequence for PRO299.

The entire nucleotide sequence of UNQ262 (DNA39976-1215) is shown in Figure 8 (SEQ ID NO:14). Clone UNQ262 (DNA39976-1215) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 111-113 and ending at the stop codon at nucleotide positions 2322-2324 (Figure 8). The predicted polypeptide precursor is 737 amino acids long (Figure 9). Important regions of the polypeptide sequence encoded by clone UNQ262 (DNA39976-1215) have been identified and include the following: a signal peptide corresponding to amino acids 1-28, a putative transmembrane region corresponding to amino acids 638-662, 10 EGF repeats, corresponding to amino acids 80-106, 121-203, 336-360, 378-415, 416-441, 454-490, 491-528, 529-548, 567-604, and 605-622, respectively, and 10 potential N-glycosylation sites, corresponding to amino acids 107-120, 204-207, 208-222, 223-285, 286-304, 361-374, 375-377, 442-453, 549-563, and 564-566, respectively. Clone UNQ262 (DNA39976-1215) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209524.

Analysis of the amino acid sequence of the full-length PRO299 polypeptide suggests that portions of it possess significant homology to the notch protein, thereby indicating that PRO299 may be a novel notch protein homolog and have activity typical of the notch protein.

30 EXAMPLE 7: Isolation of cDNA Clones Encoding Human PRO323

A consensus DNA sequence was assembled relative to other EST sequences as described in Example 1 above. This consensus sequence is herein designated DNA30875. Based on the DNA30875 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO323.

35 PCR primers (two forward and one reverse) were synthesized:

forward PCR primer 1 5'-AGTTCTGGTCAGCCTATGTGCC-3' (SEQ ID NO:25)

forward PCR primer 2 5'-CGTGATGGTGTCTTTGTCCATGGG-3' (SEQ ID NO:26)

reverse PCR primer 5'-CTCCACCAATCCCAGATGAAGTTGG-3' (SEQ ID NO:27)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30875 sequence which had the following nucleotide sequence

hybridization probe

5'-GAGCAGATTGACCTCATACGCCGCATGTGTGCCTCCTATTCTGAGCTGGA-3' (SEQ ID NO:11)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO323 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal liver tissue (LIB6).

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO323 [herein designated as UNQ284 (DNA35595-1228)] (SEQ ID NO:23) and the derived protein sequence for PRO323.

The entire nucleotide sequence of UNQ284 (DNA35595-1228) is shown in Figure 12 (SEQ ID NO:23). Clone UNQ284 (DNA35595-1228) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 110-112 and ending at the stop codon at nucleotide positions 1409-1411 (Figure 12). The predicted polypeptide precursor is 433 amino acids long (Figure 13). The full-length PRO323 protein shown in Figure 13 has an estimated molecular weight of about 47,787 daltons and a pI of about 6.11. Clone UNQ284 (DNA35595-1228) has been deposited with ATCC and is assigned ATCC deposit no. 209528.

Analysis of the amino acid sequence of the full-length PRO323 polypeptide suggests that portions of it possess significant homology to various dipeptidase proteins, thereby indicating that PRO323 may be a novel dipeptidase protein.

EXAMPLE 8: Isolation of cDNA Clones Encoding Human PRO327

An expressed sequence tag (EST) DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched and various EST sequences were identified which showed certain degrees of homology to human prolactin receptor protein. Those EST sequences were aligned using phrap and a consensus sequence was obtained. This consensus DNA sequence was then extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. The extended assembly sequence is herein designated DNA38110. The above searches were performed using the computer program BLAST or BLAST2 (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington; <http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>).

Based upon the DNA38110 consensus sequence obtained as described above, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO327.

PCR primers (forward and reverse) were synthesized as follows:

forward PCR primer 5'-CCCCCCCCGACGTGCACGTGAGCC-3' (SEQ ID NO:33)

reverse PCR primer 5'-TGAGCCAGCCCAGGAAGTCTTG-3' (SEQ ID NO:34)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA38110 consensus sequence which had the following nucleotide sequence

hybridization probe

5'-CAAGTGCCTGCAACCCCTTTGGCATCTATGGCTCCAAGAAAGCCGGGAT-3' (SEQ ID NO:35)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO327 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal lung tissue (LIB26).

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO327 [herein designated as UNQ288 (DNA38113-1230)] (SEQ ID NO:16) and the derived protein sequence for PRO327.

The entire nucleotide sequence of UNQ288 (DNA38113-1230) is shown in Figure 16 (SEQ ID NO:31). Clone UNQ288 (DNA38113-1230) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 119-121 and ending at the stop codon at nucleotide positions 1385-1387 (Figure 16). The predicted polypeptide precursor is 422 amino acids long (Figure 17). The full-length PRO327 protein shown in Figure 17 has an estimated molecular weight of about 46,302 daltons and a pI of about 9.42. Clone UNQ288 (DNA38113-1230) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209530.

Analysis of the amino acid sequence of the full-length PRO327 polypeptide suggests that it possess significant homology to the human prolactin receptor protein, thereby indicating that PRO327 may be a novel prolactin binding protein.

EXAMPLE 9: Isolation of cDNA Clones Encoding Human PRO233

A consensus DNA sequence was assembled relative to other EST sequences as described in Example 1 above. This consensus sequence is herein designated DNA30945. Based on the DNA30945 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO233.

PCR primers were synthesized as followed:

forward PCR primer 5'-GGTGAAGGCAGAAATTGGAGATG-3' (SEQ ID NO:38)

reverse PCR primer 5'-ATCCCATGCATCAGCCTGTTTACC-3' (SEQ ID NO:39)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30945 sequence which had the following nucleotide sequence

hybridization probe

5'-GCTGGTGTAGTCTATACATCAGATTTGTTTGCTACACAAGATCCTCAG-3'

(SEQ ID NO:40)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO233 gene using the probe oligonucleotide. RNA for construction of the cDNA libraries was isolated from human fetal brain tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO233 [herein designated as UNQ207 (DNA34436-1238)] (SEQ ID NO:36) and the derived protein sequence for PRO233.

The entire nucleotide sequence of UNQ207 (DNA34436-1238) is shown in Figure 18 (SEQ ID NO:36). Clone UNQ207 (DNA34436-1238) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 101-103 and ending at the stop codon at nucleotide positions 1001-1003 (Figure 18). The

predicted polypeptide precursor is 300 amino acids long (Figure 19). The full-length PRO233 protein shown in Figure 19 has an estimated molecular weight of about 32,964 daltons and a pI of about 9.52. In addition, regions of interest including the signal peptide and a putative oxidoreductase active site, are designated in Figure 19. Clone UNQ207 (DNA34436-1238) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209523

Analysis of the amino acid sequence of the full-length PRO233 polypeptide suggests that portions of it possess significant homology to various reductase proteins, thereby indicating that PRO233 may be a novel reductase.

EXAMPLE 10: Isolation of cDNA Clones Encoding Human PRO344

A consensus DNA sequence was assembled relative to other EST sequences as described in Example 1 above. This consensus sequence is herein designated DNA34398. Based on the DNA34398 consensus sequences, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO344.

Based on the DNA34398 consensus sequence, forward and reverse PCR primers were synthesized as follows:

forward PCR primer	(34398.f1)	5'-TACAGGCCCCAGTCAGGACCAGGGG-3'	(SEQIDNO:43)
forward PCR primer	(34398.f2)	5'-AGCCAGCCTCGCTCTCGG-3'	(SEQIDNO:44)
forward PCR primer	(34398.f3)	5'-GTCTGCGATCAGGTCTGG-3'	(SEQIDNO:45)
reverse PCR primer	(34398.r1)	5'-GAAAGAGGCAATGGATTTCGC-3'	(SEQIDNO:46)
reverse PCR primer	(34398.r2)	5'-GACTTACACTTGCCAGCACAGCAC-3'	(SEQIDNO:47)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the DNA34398 consensus sequence which had the following nucleotide sequence

hybridization probe	(34398.p1)	5'-GGAGCACCACTGGAGGGTCCGGAGTAGCGAGCGCCCCGAAG-3'	(SEQIDNO:48)
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In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO344 genes using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO344 [herein designated as UNQ303 (DNA40592-1242)] (SEQ ID NO:41) and the derived protein sequence for PRO344.

The entire nucleotide sequence of UNQ303 (DNA40592-1242) is shown in Figure 20 (SEQ ID NO:41). Clone UNQ303 (DNA40592-1242) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 227-229 and ending at the stop codon at nucleotide positions 956-958 (Figure 20). The predicted polypeptide precursor is 243 amino acids long (Figure 21). Important regions of the amino acid sequence encoded by nucleotides 1 to 729 of PRO344 include the signal peptide, the start of the mature protein, and two potential N-myristoylation sites as shown in Figure 21. Clone UNQ303 (DNA40592-1242) has been deposited with the ATCC and is assigned ATCC deposit no. ATCC 209492

Analysis of the amino acid sequence of the full-length PRO344 polypeptides suggests that portions of them possess significant homology to various human and murine complement proteins, thereby indicating that PRO344 may be a novel complement protein.

EXAMPLE 11: Isolation of cDNA Clones Encoding Human PRO347

A consensus DNA sequence was assembled relative to other EST sequences as described in Example 1 above. This consensus sequence is herein designated DNA39499. Based on the DNA39499 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO347.

PCR primers (forward and reverse) were synthesized as follows:

forward PCR primer 5'-AGGAACTTCTGGATCGGGCTCACC-3' (SEQ ID NO:51)

reverse PCR primer 5'-GGGTCTGGGCCAGGTGGAAGAGAG-3' (SEQ ID NO:52)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA39499 sequence which had the following nucleotide sequence

hybridization probe

5'-GCCAAGGACTCCTTCGCTGGGCCACAGGGGAGCACCAGGCCTTC-3' (SEQ ID NO:53)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO347 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (LIB228).

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO347 [herein designated as UNQ306 (DNA44176-1244)] (SEQ ID NO:49) and the derived protein sequence for PRO347.

The entire nucleotide sequence of UNQ306 (DNA44176-1244) is shown in Figure 22 (SEQ ID NO:49). Clone UNQ306 (DNA44176-1244) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 123-125 and ending at the stop codon at nucleotide positions 1488-1490 (Figure 22). The predicted polypeptide precursor is 455 amino acids long (Figure 23). The full-length PRO347 protein shown in Figure 23 has an estimated molecular weight of about 50,478 daltons and a pI of about 8.44. Clone UNQ306 (DNA44176-1244) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209532

Analysis of the amino acid sequence of the full-length PRO347 polypeptide suggests that portions of it possess significant homology to various cysteine-rich secretory proteins, thereby indicating that PRO347 may be a novel cysteine-rich secretory protein.

EXAMPLE 12: Isolation of cDNA Clones Encoding Human PRO354

An expressed sequence tag (EST) DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched and various EST sequences were identified which possessed certain degree of homology with the inter-alpha-trypsin inhibitor heavy chain and with one another. Those homologous EST sequences were then aligned and a consensus sequence was obtained. The obtained consensus DNA sequence was then extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using homologous EST sequences derived from both public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The extended assembly sequence is herein designated DNA39633. The above searches were performed using the computer program BLAST or BLAST2 (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington;

<http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>).

Based on the DNA39633 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO354. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

PCR primers were synthesized as follows:

forward PCR primer 1 (39633.f1) 5'-GTGGGAACCAAACCTCCGGCAGACC-3' (SEQ ID NO:56)

forward PCR primer 2 (39633.f2) 5'-CACATCGAGCGTCTCTGG-3' (SEQ ID NO:57)

reverse PCR primer (39633.r1) 5'-AGCCGCTCCTTCTCCGGTTCATCG-3' (SEQ ID NO:58)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA39633 sequence which had the following nucleotide sequence

hybridization probe

5'-TGGAAGGACCACTTGATATCAGTCACTCCAGACAGCATCAGGGATGGG-3' (SEQ ID NO:59)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO354 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (LIB227). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to Sall hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO354 [herein designated as UNQ311 (DNA44192-1246)] (SEQ ID NO:54) and the derived protein sequence for PRO354.

The entire nucleotide sequence of UNQ311 (DNA44192-1246) is shown in Figure 24 (SEQ ID NO:54). Clone UNQ311 (DNA44192-1246) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 72-74 and ending at the stop codon at nucleotide positions 2154-2156 (Figure 24). The predicted polypeptide precursor is 694 amino acids long (Figure 25). The full-length PRO354 protein shown in Figure 25 has an estimated molecular weight of about 77,400 daltons and a pI of about 9.54. Clone UNQ311 (DNA44192-1246) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209531.

Analysis of the amino acid sequence of the full-length PRO354 polypeptide suggests that it possess significant homology to the inter-alpha-trypsin inhibitor heavy chain protein, thereby indicating that PRO354 may be a novel inter-alpha-trypsin inhibitor heavy chain protein homolog.

EXAMPLE 13: Isolation of cDNA Clones Encoding Human PRO355

A consensus DNA sequence was assembled relative to other EST sequences using BLAST and phrap as described in Example 1 above. This consensus sequence is herein designated DNA35702. Based on the DNA35702 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO355.

Forward and reverse PCR primers were synthesized as follows:

<u>forward PCR primer</u> (.f1)	5'-GGCTTCTGCTGTTGCTCTTCTCCG-3'	(SEQ ID NO:62)
<u>forward PCR primer</u> (.f2)	5'-GTACACTGTGACCAGTCAGC-3'	(SEQ ID NO:63)
<u>forward PCR primer</u> (.f3)	5'-ATCATCACAGATTCCCGAGC-3'	(SEQ ID NO:64)
<u>reverse PCR primer</u> (.r1)	5'-TTCAATCTCCTCACCTTCCACCGC-3'	(SEQ ID NO:65)
<u>reverse PCR primer</u> (.r2)	5'-ATAGCTGTGTCTGCGTCTGCTGCG-3'	(SEQ ID NO:66)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA35702 sequence which had the following nucleotide sequence:

hybridization probe

5'-CGCGGCACTGATCCCCACAGGTGATGGGCAGAACTGTGTTACGAAAGACG-3' (SEQ ID NO:67)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO355 gene using the probe oligonucleotide. RNA for construction of the cDNA libraries was isolated from human fetal liver tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO355 [herein designated as UNQ312 (DNA39518-1247)] (SEQ ID NO:60) and the derived protein sequence for PRO355.

The entire nucleotide sequence of UNQ312 (DNA39518-1247) is shown in Figure 26 (SEQ ID NO:60). Clone UNQ312 (DNA39518-1247) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 22-24 and ending at the stop codon at nucleotide positions 1342-1344 (Figure 26). The predicted polypeptide precursor is 440 amino acids long (Figure 27). The full-length PRO355 protein shown in Figure 27 has an estimated molecular weight of about 48,240 daltons and a pI of about 4.93. In addition, regions of interest including the signal peptide, Ig repeats in the extracellular domain, potential N-glycosylation sites, and the potential transmembrane domain, are designated in Figure 27. Clone UNQ312 (DNA39518-1247) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209529.

Analysis of the amino acid sequence of the full-length PRO355 polypeptide suggests that portions of it possess significant homology to the CRTAM protein, thereby indicating that PRO355 may be CRTAM protein.

EXAMPLE 14: Isolation of cDNA Clones Encoding Human PRO357

The sequence expression tag clone no. "2452972" by Incyte Pharmaceuticals, Palo Alto, CA was used to begin a data base search. The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases which overlapped with a portion of Incyte EST clone no. "2452972". The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6

frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington; <http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>).

A consensus DNA sequence was then assembled relative to other EST sequences using phrap. This consensus sequence is herein designated DNA37162. In this case, the consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above.

Based on the DNA37162 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO357. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., *Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

PCR primers were synthesized as follows:

forward primer 1: 5'-CCCTCCACTGCCCCACCGACTG-3' (SEQ ID NO:70);

reverse primer 1: 5'-CGGTTCTGGGGACGTTAGGGCTCG-3' (SEQ ID NO:71); and

forward primer 2: 5'-CTGCCCACCGTCCACCTGCCTCAAT-3' (SEQ ID NO:72).

Additionally, two synthetic oligonucleotide hybridization probes were constructed from the consensus DNA37162 sequence which had the following nucleotide sequences:

hybridization probe 1:

5'-AGGACTGCCCACCGTCCACCTGCCTCAATGGGGGCACATGCCACC-3' (SEQ ID NO:73); and

hybridization probe 2:

5'-ACGCAAAGCCCTACATCTAAGCCAGAGAGACAGGGCAGCTGGG-3' (SEQ ID NO:74).

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with a PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO357 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal liver tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO357 [herein designated as UNQ314 (DNA44804-1248)] (SEQ ID NO:68) and the derived protein sequence for PRO357.

The entire nucleotide sequence of UNQ314 (DNA44804-1248) is shown in Figure 28 (SEQ ID NO:68). Clone UNQ314 (DNA44804-1248) contains a single open reading frame with an apparent translational initiation site

at nucleotide positions 137-139 and ending at the stop codon at nucleotide positions 1931-1933 (Figure 28). The predicted polypeptide precursor is 598 amino acids long (Figure 29). Clone UNQ314 (DNA44804-1248) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209527

Further analysis shows a number of characteristics as shown in Figure 29. Figure 29 shows the amino acid sequence (SEQ ID NO:69) derived from nucleotides 137 through 1930 of SEQ ID NO:68. Molecular weight is 63,030 daltons; pI is 7.24; and NX(S/T) is 3. The putative transmembrane domain is shown in Figure 29 at amino acids 506 through 524. Alternatively, the transmembrane region begins with the "G" at amino acid 497. The potential N-glycosylation sites are underlined in Figure 29. The EGF-like domain cysteine pattern signature appears at amino acids 355 through 366. This region can also be found in milk fat globule protein from rat, notch or the hepatocyte growth factor converting protease. The signal peptide is also at amino acids 1-22 of Figure 29. The start of the homology to ALS and other leucine-repeat rich proteins in the extracellular domain begins at amino acid position 24.

Analysis of the amino acid sequence of the full-length PRO357 polypeptide therefore suggests that portions of it possess significant homology to ALS, thereby indicating that PRO357 may be a novel leucine rich repeat protein related to ALS.

EXAMPLE 15: Isolation of cDNA Clones Encoding Human PRO715

A proprietary EST DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched for EST sequences encoding polypeptides having homology to human TNF-α. This search resulted in the identification of Incyte Expressed Sequence Tag No. 2099855.

A consensus DNA sequence was then assembled relative to other EST sequences using seqext and "phrap" (Phil Green, University of Washington, Seattle, Washington; <http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>). This consensus sequence is herein designated DNA52092. Based upon the alignment of the various EST clones identified in this assembly, a single EST clone from the Merck/Washington University EST set (EST clone no. 725887, Accession No. AA292358) was obtained and its insert sequenced. The full-length DNA52722-1229 sequence was then obtained from sequencing the insert DNA from EST clone no. 725887.

The entire nucleotide sequence of UNQ383 (DNA52722-1229) is shown in Figure 30 (SEQ ID NO:75). Clone UNQ383 (DNA52722-1229) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 114-116 and ending at the stop codon at nucleotide positions 864-866 (Figure 30). The predicted polypeptide is 250 amino acids long (Figure 31). The full-length PRO715 protein shown in Figure 31 has an estimated molecular weight of about 27,433 daltons and a pI of about 9.85.

Analysis of the amino acid sequence of the full-length PRO715 polypeptide suggests that it possesses significant homology to members of the tumor necrosis factor family of proteins, thereby indicating that PRO715 is a novel tumor necrosis factor protein.

EXAMPLE 16: Isolation of cDNA Clones Encoding Human PRO353

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is herein designated DNA36363. The consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the

sources of EST sequences discussed above. Based on the DNA36363 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO353.

Based on the DNA36363 consensus sequence, forward and reverse PCR primers were synthesized as follows:

- 5 forward PCR primer (36363.f1) 5'-TACAGGCCCGAGTCAGGACCAGGGG-3' (SEQIDNO:87)
reverse PCR primer (36363.r1) 5'-CTGAAGAAGTAGAGGCCGGGCACG-3' (SEQIDNO:88).

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the DNA36363 consensus sequence which had the following nucleotide sequence:

hybridization probe 36363.p1

- 10 5'-CCCGGTGCTTGCGCTGCTGTGACCCCGGTACCTCCATGTACCCGG-3' (SEQIDNO:89)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO353 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue.

- 15 DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO353 [herein designated as UNQ310 (DNA41234-1242)] (SEQ ID NO:85) and the derived protein sequence for PRO353.

The entire nucleotide sequence of UNQ310 (DNA41234-1242) is shown in Figure 34 (SEQ ID NO:85). Clone UNQ310 (DNA41234-1242) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 305-307 and ending at the stop codon at nucleotide positions 1148-1150 (Figure 34). The predicted polypeptide precursor is 281 amino acids long (Figure 35). Important regions of the amino acid sequence encoded by PRO353 include the signal peptide, corresponding to amino acids 1-26, the start of the mature protein at amino acid position 27, a potential N-glycosylation site, corresponding to amino acids 93-98 and a region which has homology to a 30 kd adipocyte complement-related protein precursor, corresponding to amino acids 99-281. Clone UNQ310 (DNA41234-1242) has been deposited with the ATCC and is assigned ATCC deposit no. ATCC

- 25 209618

Analysis of the amino acid sequence of the full-length PRO353 polypeptides suggests that portions of them possess significant homology to portions of human and murine complement proteins, thereby indicating that PRO353 may be a novel complement protein.

30 EXAMPLE 17: Isolation of cDNA Clones Encoding Human PRO361

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is herein designated DNA40654. Based on the DNA40654 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO361.

- 35 Forward and reverse PCR primers were synthesized as follows:

forward PCR primer (.f1) 5'-AGGGAGGATTATCCTTGACCTTTGAAGACC-3' (SEQ ID NO:92)
forward PCR primer (.f2) 5'-GAAGCAAGTGCCCGAGCTC-3' (SEQ ID NO:93)
forward PCR primer (.f3) 5'-CGGGTCCCTGCTCTTTGG-3' (SEQ ID NO:94)
reverse PCR primer (.r1) 5'-CACCGTAGCTGGGAGCGCACTCAC-3' (SEQ ID NO:95)

reverse PCR primer (.r2) 5'-AGTGTAAGTCAAGCTCCC-3'

(SEQ ID NO:96)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA40654 sequence which had the following nucleotide sequence

hybridization probe

5'- GCTTCCTGACACTAAGGCTGTCTGCTAGTCAGAATTGCCTCAAAAAGAG-3'

(SEQ ID NO:97)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO361 gene using the probe oligonucleotide. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO361 [herein designated as UNQ316 (DNA45410-1250)] (SEQ ID NO:90) and the derived protein sequence for PRO361.

The entire nucleotide sequence of UNQ316 (DNA45410-1250) is shown in Figure 36 (SEQ ID NO:90). Clone UNQ316 (DNA45410-1250) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 226-228 and ending at the stop codon at nucleotide positions 1519-1521 (Figure 36). The predicted polypeptide precursor is 431 amino acids long (Figure 37). The full-length PRO361 protein shown in Figure 37 has an estimated molecular weight of about 46,810 daltons and a pI of about 6.45. In addition, regions of interest including the transmembrane domain (amino acids 380-409) and sequences typical of the arginase family of proteins (amino acids 3-14 and 39-57) are designated in Figure 37. Clone UNQ316 (DNA45410-1250) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209621.

Analysis of the amino acid sequence of the full-length PRO361 polypeptide suggests that portions of it possess significant homology to the mucin and/or chitinase proteins, thereby indicating that PRO361 may be a novel mucin and/or chitinase protein.

EXAMPLE 18: Isolation of cDNA Clones Encoding Human PRO365

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is herein designated DNA35613. Based on the DNA35613 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO365.

Forward and reverse PCR primers were synthesized as follows:

forward PCR primer (.f1-35613) 5'-AATGTGACCACTGGACTCCC-3'

(SEQ ID NO:100)

forward PCR primer (.f2-35613) 5'-AGGCTTGGAACCTCCCTTC-3'

(SEQ ID NO:101)

reverse PCR primer (.r1-35613) 5'-AAGATTCTTGAGCGATTCCAGCTG-3'

(SEQ ID NO:102)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA35613 sequence which had the following nucleotide sequence

hybridization probe

5'-AATCCCTGCTCTTCATGGTGACCTATGACGACGGAAGCACAAGACTG-3'

(SEQ ID NO:103)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO365 gene using the probe oligonucleotide and one of the PCR primers. RNA for

construction of the cDNA libraries was isolated from human fetal kidney tissue.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO365 [herein designated as UNQ320 (DNA46777-1253)] (SEQ ID NO:98) and the derived protein sequence for PRO365.

~~The entire nucleotide sequence of UNQ320 (DNA46777-1253) is shown in Figure 38 (SEQ ID NO:98).~~

Clone UNQ320 (DNA46777-1253) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 15-17 and ending at the stop codon at nucleotide positions 720-722 (Figure 38). The predicted polypeptide precursor is 235 amino acids long (Figure 39). Important regions of the polypeptide sequence encoded by Clone UNQ320 (DNA46777-1253) have been identified and include the following: a signal peptide corresponding to amino acids 1-20, the start of the mature protein corresponding to amino acid 21, and multiple potential N-glycosylation sites as shown in Figure 39. Clone UNQ320 (DNA46777-1253) has been deposited with ATCC and is assigned ATCC deposit no. ATCC 209619.

Analysis of the amino acid sequence of the full-length PRO365 polypeptide suggests that portions of it possess significant homology to the human 2-19 protein, thereby indicating that PRO365 may be a novel human 2-19 protein homolog.

15 EXAMPLE 19: Use of PRO Polypeptide-Encoding Nucleic Acid as Hybridization Probes

The following method describes use of a nucleotide sequence encoding a PRO polypeptide as a hybridization probe.

DNA comprising the coding sequence of a PRO polypeptide of interest as disclosed herein may be employed as a probe or used as a basis from which to prepare probes to screen for homologous DNAs (such as those encoding naturally-occurring variants of the PRO polypeptide) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO polypeptide-encoding nucleic acid-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO polypeptide can then be identified using standard techniques known in the art.

30 EXAMPLE 20: Expression of PRO Polypeptides in *E. coli*

This example illustrates preparation of an unglycosylated form of a desired PRO polypeptide by recombinant expression in *E. coli*.

The DNA sequence encoding the desired PRO polypeptide is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase

cleavage site), the specific PRO polypeptide coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO polypeptide can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

PRO241 was successfully expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding PRO241 was initially amplified using selected PCR primers. The primers contained restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences were then ligated into an expression vector, which was used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq). Transformants were first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 was reached. Cultures were then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH₄)₂SO₄, 0.71 g sodium citrate·2H₂O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycaze SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO₄) and grown for approximately 20-30 hours at 30°C with shaking. Samples were removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets were frozen until purification and refolding.

E. coli paste from 0.5 to 1 L fermentations (6-10 g pellets) was resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution was stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution was centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant was diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. Depending the clarified extract was loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column was washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein was eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein were pooled and stored at 4°C. Protein concentration was estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins were refolded by diluting sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes were chosen so that the final protein concentration was between 50 to 100 micrograms/ml. The refolding solution was stirred gently at 4°C for 12-36 hours. The refolding reaction was quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution was

filtered through a 0.22 micron filter and acetonitrile was added to 2-10% final concentration. The refolded protein was chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance were analyzed on SDS polyacrylamide gels and fractions containing homogeneous refolded protein were pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded PRO241 protein were pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins were formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

EXAMPLE 21: Expression of PRO Polypeptides in Mammalian Cells

This example illustrates preparation of a glycosylated form of a desired PRO polypeptide by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO polypeptide-encoding DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO polypeptide DNA using ligation methods such as described in Sambrook et al., *supra*. The resulting vector is called pRK5-PRO polypeptide.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 μ g pRK5-PRO polypeptide DNA is mixed with about 1 μ g DNA encoding the VA RNA gene [Thimmappaya et al., *Cell*, 31:543 (1982)] and dissolved in 500 μ l of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl_2 . To this mixture is added, dropwise, 500 μ l of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO_4 , and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 μ Ci/ml ^{35}S -cysteine and 200 μ Ci/ml ^{35}S -methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO polypeptide may be introduced into 293 cells transiently using the dextran sulfate method described by Sompariyac et al., *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 μ g pRK5-PRO polypeptide DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium,

and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO polypeptide can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO polypeptides can be expressed in CHO cells. The pRK5-PRO polypeptide can be transfected into CHO cells using known reagents such as CaPO₄ or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ³⁵S-methionine. After determining the presence of PRO polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO polypeptide can then be concentrated and purified by any selected method.

Epitope-tagged PRO polypeptide may also be expressed in host CHO cells. The PRO polypeptide may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO polypeptide insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO polypeptide can then be concentrated and purified by any selected method, such as by Ni²⁺-chelate affinity chromatography.

PRO241 was successfully expressed in CHO cells by both a transient and a stable expression procedure. In addition, PRO243, PRO323 and PRO233 were successfully transiently expressed in CHO cells.

Stable expression in CHO cells was performed using the following procedure. The proteins were expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins were fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs were subcloned in a CHO expression vector using standard techniques as described in Ausubel et al., *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas et al., *Nucl. Acids Res.* 24: 9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA were introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect[®] (Quiagen), Dospert[®] or Eugene[®] (Boehringer Mannheim). The cells were grown and described in Lucas et al., supra. Approximately 3 x 10⁷ cells are frozen in an ampule for further growth and production as described below.

The ampules containing the plasmid DNA were thawed by placement into water bath and mixed by vortexing. The contents were pipetted into a centrifuge tube containing 10 mLs of media and centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated and the cells were resuspended in 10 mL of selective media (0.2 µm filtered PS20 with 5% 0.2 µm diafiltered fetal bovine serum). The cells were then aliquoted into a 100 mL spinner

containing 90 mL of selective media. After 1-2 days, the cells were transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, a 250 mL, 500 mL and 2000 mL spinners were seeded with 3×10^5 cells/mL. The cell media was exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in US Patent No. 5,122,469, issued June 16, 1992 was actually used. 3L production spinner is seeded at 5 1.2×10^6 cells/mL. On day 0, the cell number pH were determined. On day 1, the spinner was sampled and sparging with filtered air was commenced. On day 2, the spinner was sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion). Throughout the production, pH was adjusted as necessary to keep at around 7.2. After 10 days, or until viability dropped below 70%, the cell culture was harvested by centrifugation and filtering 10 through a 0.22 μ m filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins were purified using a Ni-NTA column (Qiagen). Before purification, imidazole was added to the conditioned media to a concentration of 5 mM. The conditioned media was pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column was washed with additional equilibration 15 buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of were purified from the conditioned media as follows. The conditioned medium was pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM 20 Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein was immediately neutralized by collecting 1 ml fractions into tubes containing 275 μ L of 1 M Tris buffer, pH 9. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity was assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

25 PRO241, PRO243, PRO299, PRO323, PRO327, PRO233, PRO344, PRO347, PRO354, PRO355, PRO357, PRO353, PRO361 and PRO365 were also successfully transiently expressed in COS cells.

EXAMPLE 22: Expression of PRO Polypeptides in Yeast

The following method describes recombinant expression of a desired PRO polypeptide in yeast.

30 First, yeast expression vectors are constructed for intracellular production or secretion of PRO polypeptides from the ADH2/GAPDH promoter. DNA encoding a desired PRO polypeptide, a selected signal peptide and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of the PRO polypeptide. For secretion, DNA encoding the PRO polypeptide can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, the yeast alpha-factor secretory signal/leader sequence, 35 and linker sequences (if needed) for expression of the PRO polypeptide.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO polypeptide can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing the PRO polypeptide may further be purified using selected column chromatography resins.

EXAMPLE 23: Expression of PRO Polypeptides in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO polypeptides in Baculovirus-infected insect cells.

The desired PRO polypeptide is fused upstream of an epitope tag contained with a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the PRO polypeptide or the desired portion of the PRO polypeptide (such as the sequence encoding the extracellular domain of a transmembrane protein) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4-5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression is performed as described by O'Reilley et al., *Baculovirus expression vectors: A laboratory Manual*, Oxford: Oxford University Press (1994).

Expressed poly-his tagged PRO polypeptide can then be purified, for example, by Ni²⁺-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., *Nature*, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl₂; 0.1 mM EDTA; 10% Glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% Glycerol, pH 7.8) and filtered through a 0.45 µm filter. A Ni²⁺-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A₂₈₀ with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% Glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A₂₈₀ baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or western blot with Ni²⁺-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His₁₀-tagged PRO polypeptide are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO polypeptide can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

PRO241, PRO327 and PRO344 were successfully expressed in baculovirus infected Sf9 insect cells. While the expression was actually performed in a 0.5-2 L scale, it can be readily scaled up for larger (e.g. 8 L) preparations. The proteins were expressed as an IgG construct (immunoadhesin), in which the protein extracellular region was fused to an IgG1 constant region sequence containing the hinge, CH2 and CH3 domains and/or in poly-His tagged forms.

For expression in baculovirus infected Sf9 cells, following PCR amplification, the respective coding sequences were subcloned into a baculovirus expression vector (pb.PH.IgG for IgG fusions and pb.PH.His.c for poly-His tagged proteins), and the vector and Baculogold® baculovirus DNA (Pharmingen) were co-transfected into 105 *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711), using Lipofectin (Gibco BRL). pb.PH.IgG and pb.PH.His are modifications of the commercially available baculovirus expression vector pVL1393 (Pharmingen), with modified polylinker regions to include the His or Fc tag sequences. The cells were grown in Hink's TNM-FH medium supplemented with 10% FBS (Hyclone). Cells were incubated for 5 days at 28°C. The supernatant was harvested and subsequently used for the first viral amplification by infecting Sf9 cells in Hink's TNM-FH medium supplemented with 10% FBS at an approximate multiplicity of infection (MOI) of 10. Cells were incubated for 3 days at 28°C. The supernatant was harvested and the expression of the constructs in the baculovirus expression vector was determined by batch binding of 1 ml of supernatant to 25 mL of Ni-NTA beads (QIAGEN) for histidine tagged proteins or Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.

The first viral amplification supernatant was used to infect a spinner culture (500 ml) of Sf9 cells grown in ESF-921 medium (Expression Systems LLC) at an approximate MOI of 0.1. Cells were incubated for 3 days at 28°C. The supernatant was harvested and filtered. Batch binding and SDS-PAGE analysis was repeated, as necessary, until expression of the spinner culture was confirmed.

The conditioned medium from the transfected cells (0.5 to 3 L) was harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein construct were purified using a Ni-NTA column (Qiagen). Before purification, imidazole was added to the conditioned media to a concentration of 5 mM. The conditioned media were pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column was washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of proteins were purified from the conditioned media as follows. The conditioned media were pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein was immediately neutralized by collecting 1 ml fractions into tubes containing 275 mL of 1 M Tris buffer, pH 9. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity of the proteins was verified by SDS polyacrylamide gel (PEG) electrophoresis and N-terminal amino acid sequencing by Edman degradation.

PRO243, PRO323, PRO344 and PRO355 were successfully expressed in baculovirus infected Hi5 insect cells. While the expression was actually performed in a 0.5-2 L scale, it can be readily scaled up for larger (e.g. 8 L) preparations.

For expression in baculovirus-infected Hi5 insect cells, the PRO polypeptide-encoding DNA may be amplified with suitable systems, such as Pfu (Stratagene), or fused upstream (5'-of) of an epitope tag contained with a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids

such as pVL1393 (Novagen). Briefly, the PRO polypeptide or the desired portion of the PRO polypeptide (such as the sequence encoding the extracellular domain of a transmembrane protein) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector. For example, derivatives of pVL1393 can include the Fc region of human IgG (pb.PH.IgG) or an 8 histidine (pb.PH.His) tag downstream (3'-of) the NAME sequence. Preferably, the vector construct is sequenced for confirmation.

Hi5 cells are grown to a confluency of 50% under the conditions of, 27°C, no CO₂, NO pen/strep. For each 150 mm plate, 30 ug of pIE based vector containing PRO polypeptide is mixed with 1 ml Ex-Cell medium (Media: Ex-Cell 401 + 1/100 L-Glu JRH Biosciences #14401-78P (note: this media is light sensitive)), and in a separate tube, 100 ul of CellFectin (CellFECTIN (GibcoBRL #10362-010) (vortexed to mix)) is mixed with 1 ml of Ex-Cell medium. The two solutions are combined and allowed to incubate at room temperature for 15 minutes. 8 ml of Ex-Cell media is added to the 2ml of DNA/CellFECTIN mix and this is layered on Hi5 cells that have been washed once with Ex-Cell media. The plate is then incubated in darkness for 1 hour at room temperature. The DNA/CellFECTIN mix is then aspirated, and the cells are washed once with Ex-Cell to remove excess CellFECTIN. 30 ml of fresh Ex-Cell media is added and the cells are incubated for 3 days at 28°C. The supernatant is harvested and the expression of the PRO polypeptide in the baculovirus expression vector can be determined by batch binding of 1 ml of supernatant to 25 mL of Ni-NTA beads (QIAGEN) for histidine tagged proteins or Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.

The conditioned media from the transfected cells (0.5 to 3 L) is harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein comprising the PRO polypeptide is purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of proteins are purified from the conditioned media as follows. The conditioned media is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275 mL of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity of PRO polypeptide can be assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation and other analytical procedures as desired or necessary.

EXAMPLE 24: Preparation of Antibodies that Bind to PRO Polypeptides

This example illustrates preparation of monoclonal antibodies which can specifically bind to a PRO polypeptide.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO polypeptide, fusion proteins containing the PRO polypeptide, and cells expressing recombinant PRO polypeptide on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO polypeptide immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO polypeptide antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO polypeptide. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against the PRO polypeptide. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against the PRO polypeptide is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO polypeptide monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

EXAMPLE 25: Chimeric PRO Polypeptides

PRO polypeptides may be expressed as chimeric proteins with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS™ extension/affinity purification system (Immunex Corp., Seattle Wash.). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen, San Diego Calif.) between the purification domain and the PRO polypeptide sequence may be useful to facilitate expression of DNA encoding the PRO polypeptide.

EXAMPLE 26: Purification of PRO Polypeptides Using Specific Antibodies

Native or recombinant PRO polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-PRO polypeptide, mature PRO polypeptide, or pre-PRO polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO polypeptide antibody to an activated

chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSE™ (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of PRO polypeptide by preparing a fraction from cells containing PRO polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble PRO polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRO polypeptide (e.g., high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/PRO polypeptide binding (e.g., a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotrope such as urea or thiocyanate ion), and PRO polypeptide is collected.

EXAMPLE 27: Drug Screening

This invention is particularly useful for screening compounds by using PRO polypeptides or binding fragment thereof in any of a variety of drug screening techniques. The PRO polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO polypeptide or a fragment and the agent being tested. Alternatively, one can examine the diminution in complex formation between the PRO polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO polypeptide-associated disease or disorder. These methods comprise contacting such an agent with an PRO polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the PRO polypeptide or fragment, or (ii) for the presence of a complex between the PRO polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the PRO polypeptide or fragment is typically labeled. After suitable incubation, free PRO polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO polypeptide or to interfere with the PRO polypeptide/cell complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO polypeptide, the peptide test compounds are reacted with

PRO polypeptide and washed. Bound PRO polypeptide is detected by methods well known in the art. Purified PRO polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO polypeptide specifically compete with a test compound for binding to PRO polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO polypeptide.

EXAMPLE 28: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (i.e., a PRO polypeptide) or of small molecules with which they interact, e.g., agonists, antagonists, or inhibitors. Any of these examples can be used to fashion drugs which are more active or stable forms of the PRO polypeptide or which enhance or interfere with the function of the PRO polypeptide *in vivo* (c.f., Hodgson, Bio/Technology, 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the PRO polypeptide, or of an PRO polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the PRO polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, Biochemistry, 31:7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, J. Biochem., 113:742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the PRO polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

EXAMPLE 29: Ability of PRO241 to Stimulate the Release of Proteoglycans from Cartilage

The ability of PRO241 to stimulate the release of proteoglycans from cartilage tissue was tested as follows.

The metacarpophalangeal joint of 4-6 month old pigs was aseptically dissected, and articular cartilage was removed by free hand slicing being careful to avoid the underlying bone. The cartilage was minced and cultured in bulk for 24 hours in a humidified atmosphere of 95% air, 5% CO₂ in serum free (SF) media (DME/F12 1:1) with

0.1% BSA and 100U/ml penicillin and 100µg/ml streptomycin. After washing three times, approximately 100 mg of articular cartilage was aliquoted into micronics tubes and incubated for an additional 24 hours in the above SF media. PRO241 polypeptides were then added at 1% either alone or in combination with 18 ng/ml interleukin-1α, a known stimulator of proteoglycan release from cartilage tissue. The supernatant was then harvested and assayed for the amount of proteoglycans using the 1,9-dimethyl-methylene blue (DMB) colorimetric assay (Farndale and Buttle, *Biochem. Biophys. Acta* 883:173-177 (1985)). A positive result in this assay indicates that the test polypeptide will find use, for example, in the treatment of sports-related joint problems, articular cartilage defects, osteoarthritis or rheumatoid arthritis.

When PRO241 polypeptides were tested in the above assay, the polypeptides demonstrated a marked ability to stimulate release of proteoglycans from cartilage tissue both basally and after stimulation with interleukin-1α and at 24 and 72 hours after treatment, thereby indicating that PRO241 polypeptides are useful for stimulating proteoglycan release from cartilage tissue.

EXAMPLE 30: *In situ* Hybridization

In situ hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis and aid in chromosome mapping.

In situ hybridization was performed following an optimized version of the protocol by Lu and Gillett, *Cell Vision* 1:169-176 (1994), using PCR-generated ³³P-labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinized in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A [³³P] UTP-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2 nuclear track emulsion and exposed for 4 weeks.

³³P-Riboprobe synthesis

6.0 µl (125 mCi) of ³³P-UTP (Amersham BF 1002, SA <2000 Ci/mmol) were speed vac dried. To each tube containing dried ³³P-UTP, the following ingredients were added:

2.0 µl 5x transcription buffer

1.0 µl DTT (100 mM)

2.0 µl NTP mix (2.5 mM : 10 µ; each of 10 mM GTP, CTP & ATP + 10 µl H₂O)

1.0 µl UTP (50 µM)

1.0 µl Rnasin

1.0 µl DNA template (1µg)

1.0 µl H₂O

1.0 µl RNA polymerase (for PCR products T3 = AS, T7 = S, usually)

The tubes were incubated at 37°C for one hour. 1.0 µl RQ1 DNase were added, followed by incubation at 37°C for 15 minutes. 90 µl TE (10 mM Tris pH 7.6/1mM EDTA pH 8.0) were added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a Microcon-50 ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, 100 µl TE were added. 1 µl of the final product was pipetted on DE81 paper and

counted in 6 ml of Biofluor II.

The probe was run on a TBE/urea gel. 1-3 μ l of the probe or 5 μ l of RNA Mrk III were added to 3 μ l of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, the sample loaded, and run at 180-250 volts for 45 minutes. The gel was wrapped in saran wrap and exposed to XAR film with an intensifying screen in -70°C freezer one hour to overnight.

5 ³²P-Hybridization

A. Pretreatment of frozen sections

The slides were removed from the freezer, placed on aluminium trays and thawed at room temperature for 5 minutes. The trays were placed in 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975 ml SQ H₂O). After deproteinization in 0.5 μ g/ml proteinase K for 10 minutes at 37°C (12.5 μ l of 10 mg/ml stock in 250 ml prewarmed RNase-free RNase buffer), the sections were washed in 0.5 x SSC for 10 minutes at room temperature. The sections were dehydrated in 70%, 95%, 100% ethanol, 2 minutes each.

B. Pretreatment of paraffin-embedded sections

The slides were deparaffinized, placed in SQ H₂O, and rinsed twice in 2 x SSC at room temperature, for 5 minutes each time. The sections were deproteinized in 20 μ g/ml proteinase K (500 μ l of 10 mg/ml in 250 ml RNase-free RNase buffer; 37°C, 15 minutes) - human embryo, or 8 x proteinase K (100 μ l in 250 ml RNase buffer, 37°C, 30 minutes) - formalin tissues. Subsequent rinsing in 0.5 x SSC and dehydration were performed as described above.

20 C. Prehybridization

The slides were laid out in a plastic box lined with Box buffer (4 x SSC, 50% formamide) - saturated filter paper. The tissue was covered with 50 μ l of hybridization buffer (3.75g Dextran Sulfate + 6 ml SQ H₂O), vortexed and heated in the microwave for 2 minutes with the cap loosened. After cooling on ice, 18.75 ml formamide, 3.75 ml 20 x SSC and 9 ml SQ H₂O were added, the tissue was vortexed well, and incubated at 42°C for 1-4 hours.

25 D. Hybridization

1.0 x 10⁶ cpm probe and 1.0 μ l tRNA (50 mg/ml stock) per slide were heated at 95°C for 3 minutes. The slides were cooled on ice, and 48 μ l hybridization buffer were added per slide. After vortexing, 50 μ l ³²P mix were added to 50 μ l prehybridization on slide. The slides were incubated overnight at 55°C.

E. Washes

30 Washing was done 2 x 10 minutes with 2xSSC, EDTA at room temperature (400 ml 20 x SSC + 16 ml 0.25M EDTA, V_f=4L), followed by RNaseA treatment at 37°C for 30 minutes (500 μ l of 10 mg/ml in 250 ml RNase buffer = 20 μ g/ml). The slides were washed 2 x 10 minutes with 2 x SSC, EDTA at room temperature. The stringency wash conditions were as follows: 2 hours at 55°C, 0.1 x SSC, EDTA (20 ml 20 x SSC + 16 ml EDTA, V_f=4L).

35 F. Oligonucleotides

In situ analysis was performed on a variety of DNA sequences disclosed herein. The oligonucleotides employed for these analyses are as follows.

(1) DNA44804-1248 (PRO357)

p1 5'-GGATTCTAATACGACTCACTATAGGGCTGCCCCGCAACCCCTTCAACTG-3' (SEQ ID NO:104)

p2 5'-CTATGAAATTAACCCTCACTAAAGGGACCGCAGCTGGGTGACCGTGTA-3' (SEQ ID NO:105)

(2) DNA52722-1229 (PRO715)

5 p1 5'-GGATTCTAATACGACTCACTATAGGGCCGCCCCGCCACCTCCT-3' (SEQ ID NO:106)

p2 5'-CTATGAAATTAACCCTCACTAAAGGGACTCGAGACACCACCTGACCCA-3' (SEQ ID NO:107)

p3 5'-GGATTCTAATACGACTCACTATAGGGCCCAAGGAAGGCAGGAGACTCT-3' (SEQ ID NO:108)

p4 5'-CTATGAAATTAACCCTCACTAAAGGGACTAGGGGGTGGGAATGAAAAG-3' (SEQ ID NO:109)

10 (3) DNA38113-1230 (PRO327)

p1 5'-GGATTCTAATACGACTCACTATAGGGCCCCCCTGAGCTCTCCCGTGTA-3' (SEQ ID NO:110)

p2 5'-CTATGAAATTAACCCTCACTAAAGGGAAGGCTCGCCACTGGTCGTAGA-3' (SEQ ID NO:111)

(4) DNA35917-1207 (PRO243)

15 p1 5'-GGATTCTAATACGACTCACTATAGGGCAAGGAGCCGGGACCCAGGAGA-3' (SEQ ID NO:112)

p2 5'-CTATGAAATTAACCCTCACTAAAGGGAGGGGGCCCTTGCTGCTGAGT-3' (SEQ ID NO:113)

G. Results

In situ analysis was performed on a variety of DNA sequences disclosed herein. The results from these analyses are as follows.

(1) DNA44804-1248 (PRO357)

Low to moderate level expression at sites of bone formation in fetal tissues and in the malignant cells of an osteosarcoma. Possible signal in placenta and cord. All other tissues negative.

Fetal tissues examined (E12-E16 weeks) include: liver, kidney, adrenals, lungs, heart, great vessels, oesophagus, stomach, spleen, gonad, brain, spinal cord and body wall.

Adult human tissues examined: liver, kidney, stomach, spleen, adrenal, pancreas, lung, colonic carcinoma, renal cell carcinoma and osteosarcoma. Acetaminophen induced liver injury and hepatic cirrhosis.

Chimp Tissues examined: thyroid, parathyroid, lymph node, nerve, tongue, thymus, adrenal, gastric mucosa and salivary gland.

30 Rhesus Monkey: cerebrum and cerebellum.

(2) DNA52722-1229 (PRO715)

Generalized high signal seen over many tissues - highest signal seen over placenta, osteoblasts, injured renal tubules, injured liver, colorectal liver metastasis and gall bladder.

35 Fetal tissues examined (E12-E16 weeks) include: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, oesophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb.

Adult human tissues examined: liver, kidney, adrenal, myocardium, aorta, spleen, lung, skin, chondrosarcoma, eye, stomach, colon, colonic carcinoma, prostate, bladder mucosa and gall bladder. Acetaminophen

induced liver injury and hepatic cirrhosis.

Rhesus Tissues examined: cerebral cortex (rm), hippocampus (rm)

Chimp Tissues examined: thyroid, parathyroid, lymph node, nerve, tongue, thymus, adrenal, gastric mucosa and salivary gland.

5 (3) DNA38113-1230 (PRO327)

High level of expression observed in developing mouse and human fetal lung. Normal human adult lung, including bronchial epithelium, was negative. Expression in submucosa of human fetal trachea, possibly in smooth muscle cells. Expression also observed in non-trophoblastic cells of uncertain histogenesis in the human placenta. In the mouse expression was observed in the developing snout and in the developing tongue. All other tissues were

10 negative. Speculated function: Probable role in bronchial development.

Fetal tissues examined (E12-E16 weeks) include: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, oesophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb.

Adult tissues examined: liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung, skin, cerebral cortex (rm), hippocampus (rm), cerebellum (rm), penis, eye, bladder, stomach, gastric carcinoma, colon, colonic carcinoma, thyroid (chimp), parathyroid (chimp) ovary (chimp) and chondrosarcoma.

(4) DNA35917-1207 (PRO243)

Cornelia de Lange syndrome (CdLS) is a congenital syndrome. That means it is present from birth. CdLS is a disorder that causes a delay in physical, intellectual, and language development. The vast majority of children with CdLS are mentally retarded, with the degree of mental retardation ranging from mild to severe. Reported IQ's from 30 to 85. The average IQ is 53. The head and facial features include small head size, thin eyebrows which often meet at the midline, long eyelashes, short upturned nose, thin downturned lips, lowset ears and high arched palate or cleft palate. Other characteristics may include language delay, even in the most mildly affected, delayed growth and small stature, low pitched cry, small hands and feet, incurved fifth fingers, simian creases, and excessive body hair. Diagnosis depends on the presence of a combination of these characteristics. Many of these characteristics appear in varying degrees. In some cases these characteristics may not be present or be so mild that they will be recognized only when observed by a trained geneticist or other person familiar with the syndrome. Although much is known about CdLS, recent reports suggest that there is much more to be learned.

30 In this study additional sections of human fetal face, head, limbs and mouse embryos were examined. No expression was seen in any of the mouse tissues. Expression was only seen with the antisense probe.

Expression was observed adjacent to developing limb and facial bones in the perosteal mesenchyme. The expression was highly specific and was often adjacent to areas undergoing vascularization. The distribution is consistent with the observed skeletal abnormalities in the Cornelia de Lange syndrome. Expression was also observed in the developing temporal and occipital lobes of the fetal brain, but was not observed elsewhere. In addition, expression was seen in the ganglia of the developing inner ear; the significance of this finding is unclear.

Though these data do not provide functional information, the distribution is consistent with the sites that are known to be affected most severely in this syndrome.

Additionally, faint expression was observed at the cleavage line in the developing synovial joint forming between the femoral head and acetabulum (hip joint). If this pattern of expression were observed at sites of joint formation elsewhere, it might explain the facial and limb abnormalities observed in the Cornelia de Lange syndrome.

EXAMPLE 31: Activity of PRO243 mRNA in Xenopus Oocytes

In order to demonstrate that the human chordin clone (DNA35917-1207) encoding PRO243 is functional and acts in a manner predicted by the *Xenopus chordin* and *Drosophila sog* genes, supercoiled plasmid DNA from DNA35917-1207 was prepared by Qiagen and used for injection into *Xenopus laevis* embryos. Micro-injection of *Xenopus chordin* mRNA into ventrovegetal blastomeres induces secondary (twinned) axes (Sasai et al., *Cell* 79:779-790 (1994)) and *Drosophila sog* also induces a secondary axis when ectopically expressed on the ventral side of the *Xenopus* embryo (Holley et al., *Nature* 376:249-253 (1995) and Schmidt et al., *Development* 121:4319-4328 (1995)). The ability of *sog* to function in *Xenopus* oocytes suggests that the processes involved in dorsoventral patterning have been conserved during evolution.

Methods

Manipulation of *Xenopus* embryos:

Adult female frogs were boosted with 200 I.U. pregnant mare serum 3 days before use and with 800 I.U. of human chorionic gonadotropin the night before injection. Fresh oocytes were squeezed out from female frogs the next morning and in vitro fertilization of oocytes was performed by mixing oocytes with minced testis from sacrificed male frogs. Developing embryos were maintained and staged according to Nieuwkoop and Faber, Normal Table of *Xenopus laevis*, N.-H. P. Co., ed. (Amsterdam, 1967).

Fertilized eggs were dejellied with 2% cysteine (pH 7.8) for 10 minutes, washed once with distilled water and transferred to 0.1 x MBS with 5% Ficoll. Fertilized eggs were lined on injection trays in 0.1 X MBS with 5% Ficoll. Two-cell stage developing *Xenopus* embryos were injected with 200 pg of pRK5 containing wild type chordin (DNA35917-1207) or 200 pg of pRK5 without an insert as a control. Injected embryos were kept on trays for another 6 hours, after which they were transferred to 0.1 X MBS with 50 mg/ml gentamycin until reaching Nieuwkoop stage 37-38.

Results:

Injection of human chordin cDNA into single blastomeres resulted in the ventralization of the tadpole. The ventralization of the tadpole is visible in the shortening and kinking of the tail and the expansion of the cement gland. The ability of human chordin to function as a ventralizing agent in *Xenopus* shows that the protein encoded by DNA35917-1207 is functional and influences dorsal-ventral patterning in frogs and suggests that the processes involved in dorsoventral patterning have been conserved during evolution, with mechanisms in common between humans, flies and frogs.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
DNA34392-1170	ATCC 209526	December 10, 1997
DNA35917-1207	ATCC 209508	December 3, 1997

	DNA39976-1215	ATCC 209524	December 10, 1997
	DNA35595-1228	ATCC 209528	December 10, 1997
	DNA38113-1230	ATCC 209530	December 10, 1997
	DNA34436-1238	ATCC 209523	December 10, 1997
	DNA40592-1242	ATCC 209492	November 21, 1997
5	DNA44176-1244	ATCC 209532	December 10, 1997
	DNA44192-1246	ATCC 209531	December 10, 1997
	DNA39518-1247	ATCC 209529	December 10, 1997
	DNA44804-1248	ATCC 209527	December 10, 1997
	DNA52722-1229	ATCC 209570	January 7, 1998
10	DNA41234-1242	ATCC 209618	February 5, 1998
	DNA45410-1250	ATCC 209621	February 5, 1998
	DNA46777-1253	ATCC 209619	February 5, 1998

These deposit were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposits will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC § 122 and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. Isolated nucleic acid having at least 80% sequence identity to a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:7), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:19), Figure 13 (SEQ ID NO:24), Figure 15 (SEQ ID NO:30), Figure 17 (SEQ ID NO:32), Figure 19 (SEQ ID NO:37), Figure 21 (SEQ ID NO:42), Figure 23 (SEQ ID NO:50), Figure 25 (SEQ ID NO:55), Figure 27 (SEQ ID NO:61), Figure 29 (SEQ ID NO:69), Figure 31 (SEQ ID NO:76), Figure 35 (SEQ ID NO:86), Figure 37 (SEQ ID NO:91), and Figure 39 (SEQ ID NO:99).
2. The nucleic acid of Claim 1, wherein said nucleotide sequence comprises a nucleotide sequence selected from the group consisting of the sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:6), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:18), Figure 12 (SEQ ID NO:23), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:31), Figure 18 (SEQ ID NO:36), Figure 20 (SEQ ID NO:41), Figure 22 (SEQ ID NO:49), Figure 24 (SEQ ID NO:54), Figure 26 (SEQ ID NO:60), Figure 28 (SEQ ID NO:68), Figure 30 (SEQ ID NO:75), Figure 34 (SEQ ID NO:85), Figure 36 (SEQ ID NO:90), and Figure 38 (SEQ ID NO:98), or the complement thereof.
3. The nucleic acid of Claim 1, wherein said nucleotide sequence comprises a nucleotide sequence selected from the group consisting of the full-length coding sequence of the sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:6), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:18), Figure 12 (SEQ ID NO:23), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:31), Figure 18 (SEQ ID NO:36), Figure 20 (SEQ ID NO:41), Figure 22 (SEQ ID NO:49), Figure 24 (SEQ ID NO:54), Figure 26 (SEQ ID NO:60), Figure 28 (SEQ ID NO:68), Figure 30 (SEQ ID NO:75), Figure 34 (SEQ ID NO:85), Figure 36 (SEQ ID NO:90), and Figure 38 (SEQ ID NO:98), or the complement thereof.
4. Isolated nucleic acid which comprises the full-length coding sequence of the DNA deposited under accession number ATCC 209526, ATCC 209508, ATCC 209524, ATCC 209528, ATCC 209530, ATCC 209523, ATCC 209492, ATCC 209532, ATCC 209531, ATCC 209529, ATCC 209527, ATCC 209570, ATCC 209618, ATCC 209621 or ATCC 209619.
5. A vector comprising the nucleic acid of Claim 1.
6. The vector of Claim 5 operably linked to control sequences recognized by a host cell transformed with the vector.
7. A host cell comprising the vector of Claim 5.
8. The host cell of Claim 7 wherein said cell is a CHO cell.
9. The host cell of Claim 7 wherein said cell is an *E. coli*.

10. The host cell of Claim 7 wherein said cell is a yeast cell.

11. A process for producing a PRO polypeptides comprising culturing the host cell of Claim 7 under conditions suitable for expression of said PRO polypeptide and recovering said PRO polypeptide from the cell culture.

5 12. Isolated native sequence PRO polypeptide having at least 80% sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:7), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:19), Figure 13 (SEQ ID NO:24), Figure 15 (SEQ ID NO:30), Figure 17 (SEQ ID NO:32), Figure 19 (SEQ ID NO:37), Figure 21 (SEQ ID NO:42), Figure 23 (SEQ ID NO:50), Figure 25 (SEQ ID NO:55), Figure 27 (SEQ ID NO:61), Figure 29 (SEQ ID NO:69), Figure 31 (SEQ ID NO:76), Figure 35 (SEQ ID NO:86), Figure 37 (SEQ ID NO:91), and Figure 39 (SEQ ID NO:99).

13. Isolated PRO polypeptide having at least 80% sequence identity to the amino acid sequence encoded by the nucleotide deposited under accession number ATCC 209526, ATCC 209508, ATCC 209524, ATCC 209528, ATCC 209530, ATCC 209523, ATCC 209492, ATCC 209532, ATCC 209531, ATCC 209529, ATCC 209527, 15 ATCC 209570, ATCC 209618, ATCC 209621 or ATCC 209619.

14. A chimeric molecule comprising a polypeptide according to Claim 12 fused to a heterologous amino acid sequence.

20 15. The chimeric molecule of Claim 14 wherein said heterologous amino acid sequence is an epitope tag sequence.

16. The chimeric molecule of Claim 14 wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

25

17. An antibody which specifically binds to a PRO polypeptide according to Claim 12.

18. The antibody of Claim 17 wherein said antibody is a monoclonal antibody.

FIGURE 1

GGACTAATCTGTGGGAGCAGTTTATTCCAGTATCACCCAGGGTGCAGCCACACCAGGACTGT
GTTGAAGGGTGTTTTTTTTCTTTTAAATGTAATACCTCCTCATCTTTTCTTCTTACACAGTG
TCTGAGAACATTTACATTATAGATAAGTAGTACATGGTGGATAACTTCTACTTTTAGGAGGA
CTACTCTCTTCTGACAGTCCTAGACTGGTCTTCTACACTAAGACACCATGAAGGAGTATGTG
CTCCTATTATTCTGGCTTTGTGCTCTGCCAAACCTTCTTTAGCCCTTCACACATCGCACT
GAAGAATATGATGCTGAAGGATATGGAAGACACAGATGATGATGATGATGATGATGATG
ATGATGATGATGAGGACAACCTCTTTTTTCCAACAAGAGAGCCAAGAAGCCATTTTTTTCCA
TTTGATCTGTTTCCAATGTGTCCATTTGGATGTCAGTGCTATTACGAGTTGTACATTGCTC
AGATTTAGGTTTGACCTCAGTCCCAACCAACATTCCATTTGATACTCGAATGCTTGATCTTC
AAAACAATAAAATTAAGGAAATCAAAGAAAATGATTTTAAAGGACTCACTTCACTTTATGGT
CTGATCCTGAACAACAACAAGCTAACGAAGATTACCCAAAAGCCTTCTAACCACAAAGAA
GTTGCGAAGGCTGTATCTGTCCACAATCAACTAAGTGAAATACCACTTAATCTTCCCAAT
CATTAGCAGAACTCAGAATTCATGAAAATAAAGTTAAGAAAATACAAAAGGACACATTCAAA
GGAATGAATGCTTTACACGTTTGGAAATGAGTGCAAACCTCCTTGATAATAATGGGATAGA
GCCAGGGGCATTTGAAGGGGTGACGGTGTTCATATCAGAATTGCAGAAGCAAAACCTGACCT
CAGTTCCCTAAAGGCTTACCACCAACTTTATTGGAGCTTCACTTAGATTATAATAAAATTTCA
ACAGTGGAACCTTGAGGATTTTAAACGATACAAAGAACTACAAAGGCTGGGCCTAGGAAACAA
CAAAATCACAGATATCGAAAATGGGAGTCTTGCTAACATACCACGTGTGAGAGAAATACATT
TGGAACAATAAACTAAAAAAATCCCTTCAGGATTACCAGAGTTGAAATACCTCCAGATA
ATCTTCCCTTCATTCTAATTCAATTGCAAGAGTGGGAGTAAATGACTTCTGTCCAACAGTGCC
AAAGATGAAGAAATCTTTATACAGTGCAATAAGTTTATTCAACAACCCGGTGAAATACTGGG
AAATGCAACCTGCAACATTTCTGTTGTGTTTTGAGCAGAATGAGTGTTCACTTGGGAACTTT
GGAATGTAATAATTAGTAATTGGTAATGTCCATTTAATATAAGATTCAAAAATCCCTACATT
TGGAATACTTGAACCTCTATTAATAATGGTAGTATTATATATACAAGCAAATATCTATTCTCA
AGTGGTAAGTCCACTGACTTATTTTATGACAAGAAATTTCAACGGAATTTTGCCAACTATT
GATACATAAGGGGTTGAGAGAAACAAGCATCTATTGCAGTTTCCTTTTTGCGTACAAATGAT
CTTACATAAATCTCATGCTTGACCATTCCCTTCTTCATAACAAAAAAGTAAGATATTCGGTA
TTTAACACTTTGTTATCAAGCACATTTTAAAAAGAACTGTACTGTAAATGGAATGCTTGACT
TAGCAAAATTTGTGCTCTTTCATTTGCTGTTAGAAAAACAGAATTAACAAAGACAGTAATGT
GAAGAGTGCATTACACTATTCTTATTCTTTAGTAACTTGGGTAGTACTGTAATATTTTTAAT
CATCTTAAAGTATGATTTGATATAATCTTATTGAAATTACCTTATCATGTCTTAGAGCCCGT
CTTTATGTTTAAAACATAATTTCTTAAAATAAAGCCTTCAGTAAATGTTCAATTACCAACTTGA
TAAATGCTACTCATAAGAGCTGGTTTGGGGCTATAGCATATGCTTTTTTTTTTTTAAATTATT
ACCTGATTTTAAAATCTCTGTAAAACGTGTAGTGTTTCATAAAATCTGTAACTCGCATTTT
AATGATCCGCTATTATAAGCTTTTAAATAGCATGAAAATTGTTAGGCTATATAACATTGCCAC
TTCAACTCTAAGGAATATTTTGGAGATATCCCTTTGGAAGACCTTGCTTGGAAGAGCCTGGA
CACTAACAATTCTACACCAAATTGTCTCTTCAAATACGTATGGACTGGATAACTCTGAGAAA
CACATCTAGTATAACTGAATAAGCAGAGCATCAAATTAACAGACAGAAACCGAAAGCTCTA
TATAAATGCTCAGAGTTCTTTATGTATTTCTTATTGGCATTCAACATATGTAAAATCAGAAA
ACAGGGAAATTTTCATTAAAAATATTGGTTTGAAAT

FIGURE 2

><maps to human chromosome 9q21-q22>

><homology to Bone/cartilage proteoglycan i precursor over length
of protein>

><signal peptide>

MKEYVLLLFLALCSA

><start mature protein>

KPFFSPSHIALKNMMLKDMEDT

><GAT repeat in cDNA - trinucleotide repeats can be associated
with repeat expansion and inherited disease>

DDDDDDDDDDDDDEDNSLFPTREPRSHFFPFDLFPMCPFGCQCYSRVVHCSDLGLTSVPTNI
PFDTRMLDLQNNKIKEIKENDFKGLTSLYGLILNKNKLTKIHPKAFLTTHKKLRR

><potential leucine zipper>

LYLSHNQ

><leucine>

LSEIPLN

><leucine>

LPKSLAE

><leucine>

LRIHENK

><valine>

VKKIQKDTFKGMNA

><leucine>

LHVLEMS

><alanine>

ANPLDNNNGIEPGAFEGVTVFHIRIAEAKLTSVPKGLPPTLLELHLDYNKISTVELEDFKRYK
ELQRLGLGNKIDIE

><potential N-glycosylation site>

NGSLANIPRVREIHLENNKLKKIPSGLPELKYLQIIFLHSNSIARVGVNDFCPTVPKMKKSL
YSAISLFNNPVKYWEMQPATFRCVLSRMSVQLGNFGM

FIGURE 3

CGGACGCGTGGGCGGACGCGTGGGCCCCGCSGCACCGCCCCGGCCCCGCCCCCTCCGCCCTCCGCACTCGC
GCCTCCCTCCCTCCGCCCCGCTCCCGCGCCCTCCTCCCTCCCTCCTCCCCAGCTGTCCCGTTTCGCGTCAT
GCCGAGCCTCCCGGCCCCCGCGGCCCCGCTGCTGCTCCTCGGGCTGCTGCTGCTCGGCTCCCGGGCCGGC
CCGCGGCGCCGGCCAGAGCCCCCGTGTGCCCCATCCGTTCTGAGAAGGAGCCGCTGCCCGTTTCGGGG
AGCGGCAGGCTGCACCTTCGGCGGGAAGGTCTATGCCTTGGACGAGACGTGGCACCCGGACCTAGGGCA
GCCATTCGGGGTGATGCGCTGCGTGCTGTGCGCCTGCGAGGCGCCTCAGTGGGGTCGCCGTACCAAGGGG
CCCTGGCAGGGTCAGCTGCAAGAACATCAAACCAGAGTGCCCAACCCCGCCTGTGGGCAGCCGCGCCA
GCTGCCGGGACACTGCTGCCAGACCTGCCCCAGGAGCGCAGCAGTTCGGAGCGGCAGCCGAGCGGCCT
GTCCTTCGAGTATCCGCGGGGACCCGGAGCATCGCAGTTATAGCGACCGCGGGGAGCCAGGCGCTGAGGA
GCGGGCCCCGTGGTGACGGCCACACGGACTTCGTGGCGCTGCTGACAGGGCCGAGGTTCGAGCGGTGGC
ACGAGCCGAGTCTCGTGTGCGCTCTAGCCTCCGCTTCTCTATCTCTACAGGCGGCTGGACCGCCCC
TACCAGGATCCGCTTCTCAGACTCCAATGGCAGTGTCTGTTTGGACACCTGCAGCCCCACCCAAGA
TGGCCTGGTCTGTGGGGTGTTGGCGGCGAGTGCTCGGTTGTCTCTGCGGCTCCTTAGGGCAGAACAGT
GCATGTGGCACTTGTGACACTCACTCACCTTCAGGGGAGGTCTGGGGGCTCTCATCCGGCACCGGGC
CCTGGCTGCAGAGACCTTCAGTGCCATCCTGACTCTAGAAGGCCCCCACAGCAGGGCGTAGGGGGCAT
CACCTTGCTCACTCTCAGTGACACAGAGGACTCCTTGCAATTTTTGCTGCTCTTCGAGGGCTGCTGGA
ACCCAGGAGTGGGGGACTAACCCAGGTTCCCTTGAGGCTCCAGATTCTACACCAGGGGCGAGCTACTGCG
AGAATTTCAGGCCAATGTCTCAGCCCAGGAACAGGCTTTGCTGAGGTGCTGCCAACCTGACAGTCCA
GGAGATGGACTGGCTGGTGCTGGGGGAGCTGACAGTGGCTGGAGTGGGAGGAGGCCAGGGCTGCG
CATCAGTGGACACATTGCTGCCAGGAAGAGCTGCGACGTCTGCAAAAGTGTCTTTGTGGGGCTGATGC
CCTGATCCAGTCCAGACGGGTGCTGCCGGCTCAGCCAGCCTCAGCTGCTAGGAAATGGCTCCCTGAT
CTATCAGGTGCAAGTGGTAGGGACAAGCAGTGAGGTGGTGGCCATGACACTGGAGACCAAGCCTCAGCG
GAGGGATCAGCGCACTGTCTGTGCCACATGGCTGGACTCCAGCCAGGAGGACACACGGCCGTGGGTAT
CTGCCCTGGGCTGGGTGCCCCAGGGGCTCATATGCTGCTGCAGAATGAGCTCTTCTGAACGTGGGCAC
CAAGGACTTCCCAGACGGAGAGCTTCGGGGGACGTGGCTGCCCTGCCCTACTGTGGGCATAGCGCCCCG
CCATGACACGTGCTCGGTGCTGGGCGGCCCCAGGAGCCCTGGCTGTACCCCTGTGAAGAGCCAAGCAGCAGG
GCACGCCCTGGCTTTCTTGGATACCCACTGTCACTGCACTATGAAGTGTGCTGGCTGGGCTTGGTGG
CTCAGAACAAGGCACTGTCACTGCCCCACCTCCTTGGGCTCCTGGAACGCCAGGGCCTCGGCGGCTGCT
GAAGGGATTCTATGGCTCAGAGGCCCCAGGGTGTGGTGAAGGACCTGGAGCCGGAATGCTGCGGCACCT
GGCAAAAGGCATGGCCTCCCTGATGATCACCACCAAGGGTAGCCCCAGAGGGGAGCTCCGAGGGCAGGT
GCACATAGCCAACCAATGTGAGGTTGGCGGACTGCGCCTGGAGGCGGCGGGGCGGAGGGGGTGCGGGC
GCTGGGGGCTCCGGATACAGCCTCTGCTGCGCGCCTGTGGTGGCTGGTCTCCCGGCCCTAGCGCCCCG
CAAACCTGGTGGTCTTGGGCGGCCCCGAGACCCCAACACATGCTTCTTCAGGGGCGAGCAGCGCCCCCA
CGGGGCTCGCTGGGCGCCCCAACTACGACCCGCTCTGCTCACTCTGCACCTGCCAGAGACGAACGGTGAT
CTGTGACCCGGTGGTGTGCCCCACCGCCAGCTGCCCCACCCGGTGCAGGCTCCCGACCACTGCTGCCC
TGTTTGCCCTGAGAAACAAGATGTGAGAGACTTGCCAGGGCTGCCAAGGAGCCGGGACCCAGGAGAGGG
CTGCTATTTTGTGGTGACCGGAGCTGGCGGGCAGCGGGTACGCGGTGGCACCCCGTTGTGCCCCCTT
TGGCTTAATTAAGTGTGCTGTCTGCACCTGCAAGGGGGGCACTGGAGAGGTGCACTGTGAGAAGGTGCA
GTGTCCCCGGCTGGCCTGTGCCAGCCTGTGCGTGTCAACCCACCGACTGCTGCAAACAGTGTCCAGT
GGGTCGGGGGCCACCCCACTGGGGGACCCCTCAGGCTGATGGGCCCCGGGGCTGCCGTTTTC
TGGGCAGTGGTTCCCAAGAGAGTCAGAGCTGGCACCCCTCAGTGCCCCCTTTTGGAGAGATGAGCTGTAT
CACCTGCAGATGTGGGGCAGGGGTGCCTCACTGTGAGCGGGATGACTGTTCACTGCCACTGTCCTGTGG
CTCGGGGAAGGAGAGTCGATGCTGTTCCCGCTGCACGGCCCCACCGCGGCCCCCAGAGACCAGAATGA
TCCAGAGCTGGAGAAAGAAGCCGAAGGCTCTTAGGGAGCAGCCAGAGGGCCAAGTGACCAAGAGGATGG
GGCTGAGCTGGGGAAGGGGTGGCATCGAGGACCTTCTTGCAATTCTCCTGTGGGAAGCCAGTGCCTTT
GCTCCTCTGTCTGCTCTACTCCCACCCCACTACCTCTGGGAACCAAGCTCCACAAGGGGGGAGAGG
CAGTGGGGCAGACCGAGGTACAGCCACTCCAAGTCTGCCCCTGCCACCTCGGCCTCTGTCTGGAA
GCCCCACCCCTTTCTCCTGTACATAATGTCACTGGCTGTTGGGATTTTAAATTTATCTTCACTCAGC
ACCAAGGGCCCCCGACACTCCACTCCTGCTGCCCTGAGCTGAGCAGAGTCATTATTGGAGAGTTTTGT
ATTTATTAAACATTTCTTTTTCAGTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIGURE 4

><subunit 1 of 1, 954 aa, 1 stop

><MW: 101960, pI: 8.21, NX(S/T): 5

MPSLPAPPAPLLLLLGLLLLGSRPARGAGPEPPVLPPIRSEKEPLPVRGAAGCTFGGKVYALDE
TWHPDLGQPFGVMRVCVLCACEAPQWGRRTGRPGRVSKNIKPECPTPACGQPRQLPGHCCQT
CPQERSSSERQPSGLSFEYPRDPEHRSYSDRGEPGAEEERARGDGHTDFVALLTGPRSQAVAR
ARVSLLRSSLRFSISYRRLDRPTRIRFSDSNGSVLFEHPAAPTQDGLVCGVWRAPVRLSLRL
LRAEQLHVALVTLTHPSGEVWGPLIRHRALAAETFSAILTLEGPPQQGVGGITLLTSLDTE
SLHFLLLFRGLLEPRSGGLTQVPLRLQILHQGQLLRELQANVSAQEPGFAEVLPNLTVQEMD
WLVLGELQMALEWAGRPGLRISGHIAARKSCDVLQSVLCGADALIPVQTGAAGSASLTLLGN
GSLIYQVQVVGTSSEVVAMTLETKPQRDQRTVLCHMAGLQPGGHTAVGICPGLGARGAHML
LQNELFLNVGTKDFPDGELRGHVAALPYCGHSARHDTLPVPLAGALVLPVKSQAAGHAWLS
LDTHCHLHYEVLLAGLGGSEQGTVTAHLLGPPGTPGPRRLKGFYGSEAQGVVKDLEPELLR
HLAKGMASLMITTKGSPRGELRGQVHIANQCEVGGLRLEAAGAEGVRALGAPDTASAAPPVV
PGLPALAPAKPGGPGRPRDPNTCFFEGQQRPHGARWAPNYDPLCSLCTCQRRTVICDPVVC
PPSCPHPVQAPDQCCPVCPEKQDVRDLPLGLPRSRDPGEGCYFDGDRSWRAAGTRWHPVVPF
GLIKCAVCTCKGGTGEVHCEKVQCPRLACAQPV RVNPTDCCKQCPVGS GAHPQLGDPMQADG
PRGCRFAGQWFPESQSWHPSVPPFGEMSCITCRGAGVPHCERDDCSLPLSCGSGKESRCCS
RCTAHRPPETRTDPELEKEAEGS

FIGURE 5

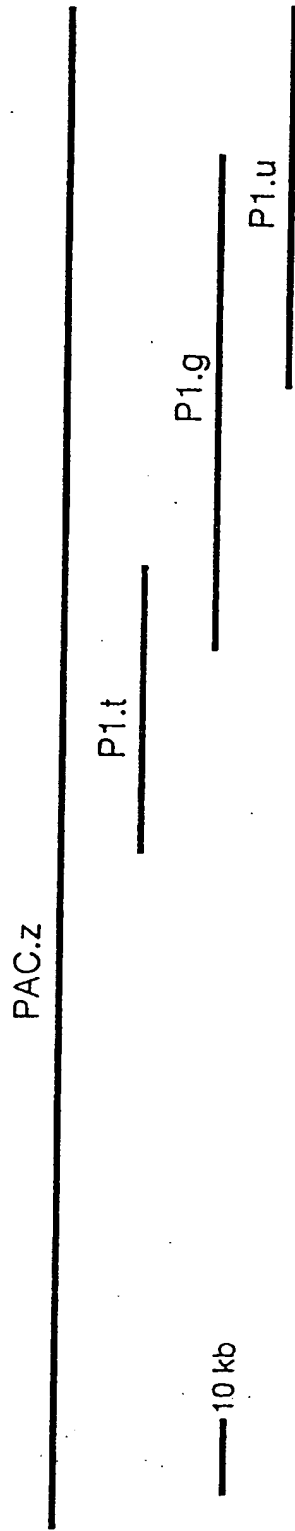


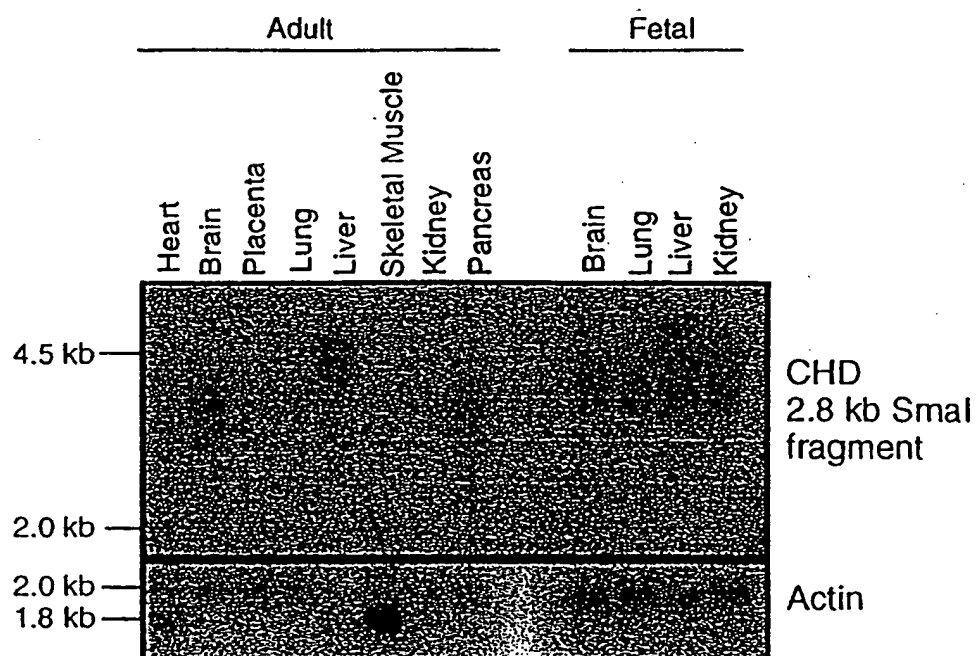
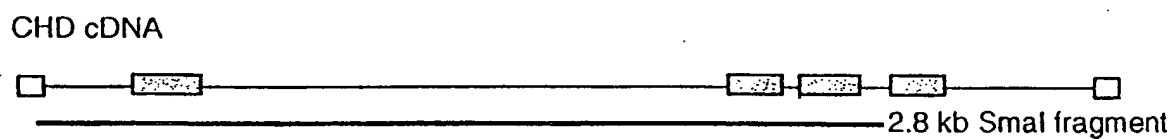
FIGURE 6**A****B**

FIGURE 7

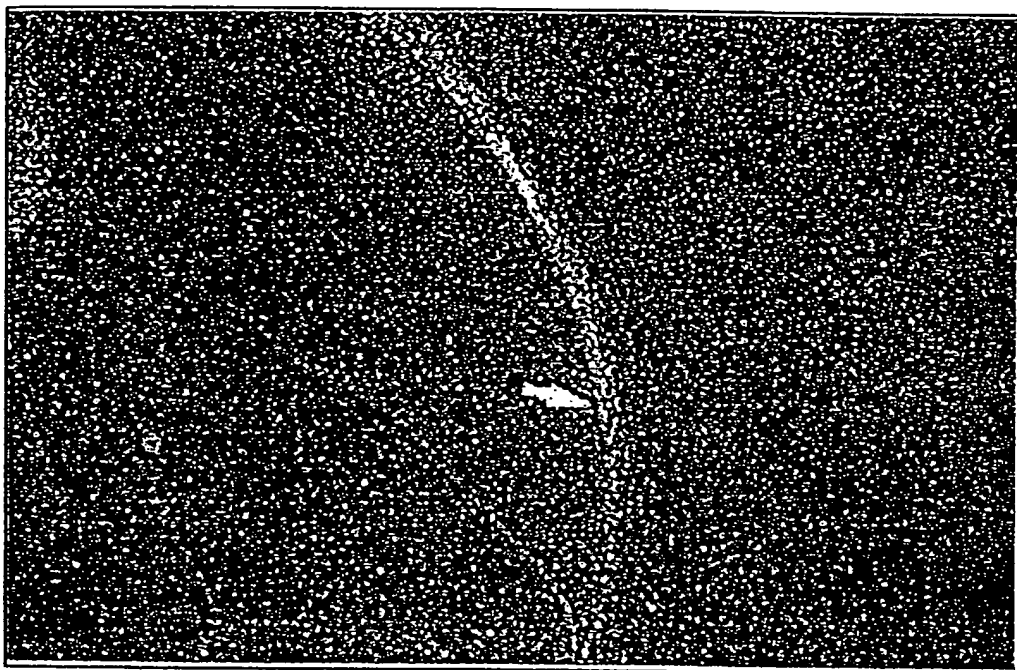


FIGURE 8

GGCGGAGCAGCCCTAGCCGCCACCGTCGCTCTCGCAGCTCTCGTCGCCACTGCCACCGCCGC
CGCCGTCACTGCGTCCTGGCTCCGGCTCCCGCGCCCTCCCGGCCGCCATGCAGCCCCGCCG
CGCCAGGCGCCCCGGTGCAGCTGCTGCCCGCGCTGGCCCTGCTGCTGCTGCTCGGAG
CGGGGCCCCGAGGCAGCTCCCTGGCCAACCCGGTGCCCGCCGCGCCCTTGTCTGCCCGGG
CCGTGCGCCGCGCAGCCCTGCCGGAATGGGGGTGTGTGCACCTCGCGCCCTGAGCCGGACCC
GCAGCACCCGGCCCCCGCCGGCGAGCCTGGCTACAGCTGCACCTGCCCCGCCGGGATCTCCG
GCGCCAACTGCCAGCTTGTTCAGATCCTTGTGCCAGCAACCCTTGTACCATGGCAACTGC
AGCAGCAGCAGCAGCAGCAGCAGCGATGGCTACCTCTGCATTTGCAATGAAGGCTATGAAGG
TCCCAACTGTGAACAGGCACTTCCCAGTCTCCAGCCACTGGCTGGACCGAATCCATGGCAC
CCCGACAGCTTCAGCCTGTTCTGCTACTCAGGAGCCTGACAAAATCCTGCCTCGCTCTCAG
GCAACGGTGACACTGCCTACCTGGCAGCCGAAAACAGGGCAGAAAGTTGTAGAAATGAAATG
GGATCAAGTGGAGGTGATCCAGATATTGCCGTGTGGGAATGCCAGTTCTAACAGCTCTGCGG
GTGGCCGCTGGTATCCTTTGAAGTGCCACAGAACACCTCAGTCAAGATTCGGCAAGATGCC
ACTGCCTCACTGATTTTGTCTGGAAGGTACGGCCACAGGATTCCAACAGTGCTCCCTCAT
AGATGGACGAAGTGTGACCCCCCTTCAGGCTTCAGGGGGACTGGTCCTCCTGGAGGAGATGC
TCGCCTTGGGGAATAATCACTTTATTGGTTTTGTGAATGATTCTGTGACTAAGTCTATTGTG
GCTTTGCGCTTAACCTCTGGTGGTGAAGGTGACACCTGTGTGCCGGGGGAGAGTCACGCAA
TGACTTGGAGTGTTCAGGAAAAGGAAAATGCACCACGAAGCCGTCAGAGGCAACTTTTTCT
GTACCTGTGAGGAGCAGTACGTGGGTACTTTCTGTGAAGAATACGATGCTTGCCAGAGGAAA
CCTTGCCAAAACAACGCGAGCTGTATTGATGCAAATGAAAAGCAAGATGGGAGCAATTCAC
CTGTGTTTGCCTTCCTGGTTATACTGGAGAGCTTTGCCAGTCCAAGATTGATTACTGCATCC
TAGACCCATGCAGAAATGGAGCAACATGCATTTCCAGTCTCAGTGGATTACCTGCCAGTGT
CCAGAAGGATACTTCGGATCTGCTTGTGAAGAAAAGGTGGACCCCTGCGCCTCGTCTCCGTG
CCAGAACAACGGCACCTGCTATGTGGACGGGGTACACTTTACCTGCAACTGCAGCCCCGGCT
TCACAGGGCCGACCTGTGCCCAGCTTATTGACTTCTGTGCCCTCAGCCCCCTGTGCTCATGGC
ACGTGCCGACGCTGGGCACCGAGCTACAAATGCCTCTGTGATCCAGGTTACCATGGCCTCTA
CTGTGAGGAGGAATATAATGAGTGCCTCTCCGCTCCATGCCTGAATGCAGCCACCTGCAGGG
ACCTCGTTAATGGCTATGAGTGTGTGTGCCTGGCAGAATACAAAGGAACACACTGTGAATTG
TACAAGGATCCCTGCGCTAACGTCAGCTGTCTGAACGGAGCCACCTGTGACAGCGACGGCCT
GAATGGCACGTGCATCTGTGCACCCGGGTTTACAGGTGAAGAGTGCACATTGACATAAATG
AATGTGACAGTAACCCCTGCCACCATGGTGGGAGCTGCCTGGACCAGCCCAATGGTTATAAC
TGCCACTGCCCGCATGGTTGGGTGGGAGCAAACCTGTGAGATCCACCTCCAATGGAAGTCCGG
GCACATGGCGGAGAGCCTACCAACATGCCACGGCACTCCCTCTACATCATCATTGGAGCCC
TCTGCGTGGCCTTCATCCTTATGCTGATCATCTGATCGTGGGGATTTGCCGCATCAGCCGC
ATTGAATACCAGGGTTCTTCCAGGCCAGCCTATGAGGAGTTCTACAACCTGCCGCAGCATCGA
CAGCGAGTTCAGCAATGCCATTGCATCCATCCGGCATGCCAGGTTTGGAAAGAAATCCCGGC
CTGCAATGTATGATGTGAGCCCCATCGCTATGAAGATTACAGTCCTGATGACAAACCCTTG
GTCACACTGATTAAACTAAAGATTTGTAATCTTTTTTTGGATTATTTTTCAAAAAGATGAG
ATACTACACTCATTTAAATATTTTTAAGAAAATAAAAAGCTTAAGAAATTTAAATGCTAGC
TGCTCAAGAGTTTTTCAGTAGAATATTTAAGAACTAATTTTCTGCAGCTTTTAGTTTGGAAAA
AATATTTTAAAAACAAAATTTGTGAAACCTATAGACGATGTTTAAATGTACCTTCAGCTCTC
TAACTGTGTGCTTCTACTAGTGTGTGCTCTTTTCACTGTAGACACTATCACGAGACCCAGA
TTAATTTCTGTGGTTGTTACAGAATAAGTCTAATCAAGGAGAAGTTTCTGTTTGACGTTTGA
GTGCCGGCTTTCTGAGTAGAGTTAGGAAAACCACGTAACGTAGCATATGATGTATAATAGAG
TATACCCGTTACTTAAAAAGAGTCTGAAATGTTTCGTTTTGTGGAAAAGAACTAGTTAAAT
TTACTATTCTTAACCCGAATGAAATTAGCCTTTGCCTTATTCTGTGCATGGGTAAGTAACTT
ATTTCTGCACTGTTTTGTGAACTTTGTGGAAACATTCTTTCGAGTTTGTTTTTGTCATTTT
CGTAACAGTCGTCGAACCTAGGCCTCAAAAACATACGTAACGAAAAGGCCCTAGCGAGGCAAAT
TCTGATTGATTTGAATCTATATTTTTCTTTAAAAAGTCAAGGGTTCTATATTGTGAGTAAAT
TAAATTTACATTTGAGTTGTTTGTGCTAAGAGGTAGTAAATGTAAGAGAGTACTGGTTCCCT
TCAGTAGTGAGTATTTCTCATAGTCAGCTTTATTTATCTCCAGGATGTTTTTGTGGCTGTA
TTTGATTGATATGTGCTTCTTCTGATTCTTGCTAATTTCCAACCATATTGAATAAATGTGAT
CAAGTCA

FIGURE 9

MQPRRAQAPGAQQLPALALLLLLLGAGPRGSSLANPVPAAPLSAPGPCAAQPCRNGGVCTSR
PEPDPQHPAPAGEPGYSCTCPAGISGANCLVADPCASNPCHHGNCSSSSSSSSDGYLCICN
EGYEGPNCEQALPSLPATGWTESMAPRQLQPVPAEQEPDKILPRSQATVTLPTWQPKTGQKV
VEMKWDQVEVIPDIACGNASSNSSAGGRLVSFEVPQNTSVKIRQDATASLILLWKVTATGFQ
QCSLIDGRSVTPLQASGGLVLEEMALGNNHFIGFVNDVTKSIVALRLTLVVKVSTCVPG
ESHANDLECSGKGKCTTKPSEATFSCTCEEQYVGTFCEEYDACQRKPCQNNASCIDANEKQD
GSNFTCVCLPGYTGELCQSKIDYCIDPCRNGATCISSLSGFTCQCPEGYFGSACEEKVDPC
ASSPCQNNGTCTYVDGVHFTCNCSPGFTGPTCAQLIDFCALSPCAHGTCRSVGTSYKCLCDPG
YHGLYCEEEYNECLSAFCLNAATCRDLVNGYECVCLA EYKGTHCELYKDPCANVSCLNGATC
DSDGLNGTCICAPGFTGEECDIDINECDSNPCHHGGSCLDQPNGYNCHCPHGWVGANCEIHL
QWKSGHMAESLTNMPRHSLYIIIGALCVAFILMLIILIVGICRISRIEYQGSSRPAYEEFYN
CRSIDSEFSNAIASIRHARFGKKS RPAMYDVSP IAYEDYSPDDKPLVTLIKTKDL

FIGURE 10

CTCTGGAAGGTCACGGCCACAGGATTCCAACAGTGCTCCCTCATAGATGGACGAAAGTGTGA
CCCCCCTTTCAGGCTTTCAGGGGGACTGGTCCTCCTGGAGGAGATGCTCGCCTTGGGGAATA
ATCACTTTATTGGTTTTGTGAATGATTCTGTGACTAAGTCTATTGTGGCTTTGCGCTTAACT
CTGGTGGTGAAGGTCAGCACCTGTGTGCCGGGGGAGAGTCACGCAAATGACTTGGAGTGTTC
AGGAAAAGGAAAATGCACCACGAAGCCGTCAGAGGCAACTTTTTCTGTACCTGTGAGGAGC
AGTACGTGGGTACTTTCTGTGAAGAATACGATGCTTGCCAGAGGAAACCTTGCCAAAACAAC
GCGAGCTGTATTGATGCAAATGAAAAGCAAGATGGGAGCAATTTACCTGTGTTTGCCTTCC
TGGTTATACTGGAGAGCTTTGCCAACCGAACTGAGATTGGAGCGAACGACCTACACCGAACT
GAGATAGGGGAG

FIGURE 11

CTCTGGAAGGTCACGGCCACAGGATTCCAACAGTGCTCCCTCATAGATGGACGAAAGTGTGA
CCCCCCTTTCAGGCTTTCAGGGGGACTGGTCCTCCTGGAGGAGATGCTCGCCTTGGGGAATA
ATCACTTTATTGGTTTTGTGAATGATTCTGTGACTAAGTCTATTGTGGCTTTGCGCTTAACT
CTGGTGGTGAAGGTCAGCACCTGTGTGCCGGGGGAGAGTCACGCAAATGACTTGGAGTGTTC
AGGAAAAGGAAAATGCACCACGAAGCCGTCAGAGGCAACTTTTTCCTGTACCTGTGAGGAGC
AGTACGTGGGTACTTTCTGTGAAGAATACGATGCTTGCCAGAGGAAACCTTGCCAAAACAAC
GCGAGCTGTATTGATGCAAATGAAAAGCAAGATGGGAGCAATTCACCTGTGTTTGCCTTCC
TGGTTATACTGGAGAGCTTTGCCAACCGAACTGAGATTGGAGCGAACGACCTACACCGAACT
GAGATAGGGGAG

FIGURE 12

GCTGAGTCTGCTGCTCCTGCTGCTGCTGCTCCAGCCTGTAACCTGTGCCTACACCACGCCAG
GCCCCCCCAGAGCCCTCACCACGCTGGGCGCCCCCAGAGCCCACACCATGCCGGGCACCTAC
GCTCCCTCGACCACACTCAGTAGTCCCAGCACCCAGGGCCTGCAAGAGCAGGCACGGGCCCT
GATGCGGGACTTCCCGCTCGTGGACGGCCACAACGACCTGCCCCCTGGTCCTAAGGCAGGTTT
ACCAGAAAGGGCTACAGGATGTTAACCTGCGCAATTTTCAGCTACGGCCAGACCAGCCTGGAC
AGGCTTAGAGATGGCCTCGTGGGCGCCAGTTCTGGTCAGCCTATGTGCCATGCCAGACCCA
GGACCGGGATGCCCTGCGCCTCACCTGGAGCAGATTGACCTCATACGCCGCATGTGTGCCT
CCTATTCTGAGCTGGAGCTTGTGACCTCGGCTAAAGCTCTGAACGACACTCAGAAATTGGCC
TGCCTCATCGGTGTAGAGGGTGGCCACTCGCTGGACAATAGCCTCTCCATCTTACGTACCTT
CTACATGCTGGGAGTGGCTACCTGACGCTCACCCACACCTGCAACACACCCTGGGCAGAGA
GCTCCGCTAAGGGCGTCCACTCCTTCTACAACAACATCAGCGGGCTGACTGACTTTGGTGAG
AAGGTGGTGGCAGAAATGAACCGCCTGGGCATGATGGTAGACTTATCCCATGTCTCAGATGC
TGTGGCACGGCGGGCCCTGGAAGTGTACAGGCACCTGTGATCTTCTCCCACTCGGCTGCCC
GGGTGTGTGCAACAGTGCTCGGAATGTTCTGATGACATCCTGCAGCTTCTGAAGAAGAAC
GGTGGCGTCGTGATGGTGTCTTTGTCCATGGGAGTAATACAGTGCAACCCATCAGCCAATGT
GTCCACTGTGGCAGATCACTTCGACCACATCAAGGCTGTCATTGGATCCAAGTTCATCGGGA
TTGGTGGAGATTATGATGGGGCCGGCAAATTCCTCAGGGGCTGGAAGACGTGTCCACATAC
CCGGTCCTGATAGAGGAGTTGCTGAGTCGTGGCTGGAGTGAGGAAGAGCTTCAGGGTGTCTCT
TCGTGGAAACCTGCTGCGGGTCTTCAGACAAGTGGAAAAGGTACAGGAAGAAAACAAATGGC
AAAGCCCCCTTGAGGACAAGTTCCTCGGATGAGCAGCTGAGCAGTTCCTGCCACTCCGACCTC
TCACGTCTGCGTCAGAGACAGAGTCTGACTTCAGGCCAGGAACCTCACTGAGATTCCCATAACA
CTGGACAGCCAAGTTACCAGCCAAGTGGTCAGTCTCAGAGTCCTCCCCCACATGGCCCCAG
TCCTTGCAAGTTGTGGCCACCTTCCAGTCCTTATTCTGTGGCTCTGATGACCCAGTTAGTCC
TGCCAGATGTCACTGTAGCAAGCCACAGACACCCACAAAGTTCCCCTGTTGTGCAGGCACA
AATATTTCTGAAATAAATGTTTTGGACATAG

FIGURE 13

><Microsomal dipeptidase by homolgy to pig gene>

><poor, if any, signal peptide>

MPGTYAPSTTLSSPSTQGLQEQARALMRDFPLVDGHNDLPLVLRQVYQKGLQDVNLR

><potential N-glycosylation site>

NFSYGQTSLDRLRDGLVGAQFWSAYVPCQTQDRDALRLTLEQIDLIRMCASYSELELVTSAKAL

><potential N-glycosylation site>

NDTQKLACLIG

><Renal dipeptidase active site>

VEGGHSLDNSLSILRTFYMLGVR

><end Renal dipeptidase active site>

YLTLTHTCNTPWAESSAKGVHSFYN

><potential N-glycosylation site>

NISGLTDFGEKVVAEMNRLGMMVDLSHVSDAVARRALEVSQAPVIFSHSAARGVCNSARNVP

DDILQLLKKNGGVVMVSLSMGVIQCNPSA

><potential N-glycosylation site>

NVSTVADHFDHIKAVIGSKFIGIGGDYDGAGKFPQGLEDVSTYPVLIEELLSRGWSEEEELQG

VLRGNLLRVFRQVEKVQEENKWQSPLEDKFPDEQLSSSCHSDLSRLRQRQSLTSGQELTEIP

IHWTAKLPAKW

><Lipid GPI-anchor>

SVSESSPHMAPVLAVVATFPVLILWL

FIGURE 14

AAAACCTATAAATATTCCGGATTATTCATACCGTCCCACCATCGGGCGCGGATCCGCGGCCG
CGAATTCTAAACCAACATGCCGGGCACCTACGCTCCCTCGACCACACTCAGTAGTCCCAGCA
CCCAGGGCCTGCAAGAGCAGGCACGGGCCCTGATGCGGGACTTCCCGCTCGTGGACGGCCAC
AACGACCTGCCCCTGGTCCTAAGGCAGGTTTACCAGAAAGGGCTACAGGATGTTAACCTGCG
CAATTTTCAGCTACGGCCAGACCAGCCTGGACAGGCTTAGAGATGGCCTCGTGGGCGCCAGT
TCTGGTCAGCCTATGTGCCATGCCAGACCCAGGACCGGGATGCCCTGCGCCTCACCTGGAG
CAGATTGACCTCATACGCCGCATGTGTGCCTCCTATTCTGAGCTGGAGCTTGTGACCTCGGC
TAAAGCTCTGAACGACACTCAGAAATTGGCCTGCCTCATCGGTGTAGAGGGTGGCCACTCGC
TGGACAATAGCCTCTCCATCTTACGTACCTTCTACATGCTGGGAGTGCCTACCTGACGCTC
ACCCACACCTGCAACACACCCTGGGCAGAGAGCTCCGCTAAGGGCGTCCACTCCTTCTACAA
CAACATCAGCGGGCTGACTGACTTTGGTGAGAAGGTGGTGGCAGAAATGAACCGCCTGGGCA
TGATGGTAGACTTATCCCATGTCTCAGATGCTGTGGCACGGCGGGCCCTGGAAGTGTACAG
GCACCTGTGATCTTCTCCCACTCGGCTGCCCGGGGTGTGTGCAACAGTGCTCGGAATGTTCC
TGATGACATCCTGCAGCTTCTGAAGAAGAACGGTGGCGTCGTGATGGTGTCTTTGTCCATGG
GAGTAATACAGTGCAACCCATCAGCCAATGTGTCCACTGTGGCAGATCACTTCGACCACATC
AAGGCTGTCAATTGGATCCAAGTTCATCGGGATTGGTGGAGATTATGATGGGGCCGGCAAATT
CCCTCAGGGGCTGGAAGACGTGTCCACATACCCGGTCCTGATAGAGGAGTTGCTGAGTCGTG
GCTGGAGTGAGGAAGAGCTTCAGGGTGTCTTTCGTGGAAACCTGCTGCGGGTCTTCAGACAA
GTGGAAAAGGTACAGGAAGAAAACAAATGGCAAAGCCCCCTTGAGGACAAGTTCCCGGATGA
GCAGCTGAGCAGTTCCTGCCACTCCGACCTCTCACGTCTGCGTCAGAGACAGAGTCTGACTT
CAGGCCAGGAACTCACTGAGATTCCCATACTGGACAGCCAAGTTACCAGCCAAGTGGTCA
GTCTCAGAGTCTTCCCCCACCCTGACAAAACCTCACACATGCCACCGTGCCCAGCACCTGA
ACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACC

FIGURE 15

></usr/seqdb2/sst/DNA/Dnaseqs.full/ss.DNA35872

><subunit 1 of 1, 446 aa, 0 stop

><NX(S/T): 5

MPGTYAPSTTLSSPSTQGLQEQARALMRDFPLVDGHNDLPLVLRQVYQKGLQDVNLRNFSYG
QTSLDRRLRDGLVGAQFWSAYVPCQTQDRDALRLTLEQIDLIRMCASYSELELVTSAKALND
TQKLACLIGVEGGHSLDNSLSILRTFYMLGVRYLTLTHTCNTPWAESSAKGVHSFYNNISGL
TDFGEKVVAEMNRLGMMVDLSHVSDAVARRALEVSQAPVIFSHSAARGVCNSARNVPDDILQ
LLKKNGGVVMVSLSMGVIQCNPSANVSTVADHFDHIKAVIGSKFIGIGGDYDGAGKFPQGLE
DVSTYPVLI EELLSRGWSEEEELQGVLRGNLLRVFRQVEKVQEENKWQSPLEDKFPDEQLSSS
CHSDLSRLRQRQSLTSGQELTEIPIHWTAKLPAKWSVSESSPHDPKTHTCPPCPAPELLGGP
SVFLFPPKPKDT

[illegible]

FIGURE 17

><signal peptide>
MPAGRRGPAAQSARRPPPLPLLLLLLCVLG
><start mature peptide>
APRAGSGAHTAVISPQDPTLLIGSSLLATCSVHGDPPGATAEGLYWTLNGRRLPPELSRVL
><potential N-glycosylation site>
NASTLALALANL
><potential N-glycosylation site>
NGSRQRSGDNLVCHARDGS
><start homolgy with PRLR_HUMAN prolactin receptor extracellular
domain>
ILAGSCLYVGLPPEKPV
><potential N-glycosylation site>
NISCWSKNMKDLTCRWTPGAHGETFLHT
><potential N-glycosylation site>
NYSLKYKLRWYGQDNTCEEYHTVGPHSCHIPKDLALFTPYEIWVEATNRLGSARSDVLTLDI
LDVVTTDPPPDVHVS RVGGLEDQLSVRWVSPALKDFLFQAKYQIRYVEDSV DWKVVDVVS
><potential N-glycosylation site>
NQTSCRLAGLKPGTVYFVQVRCNPFGIYGSKKAGI
><WSXWS Box - cytokine receptor signature>
WSEWSHPTAASP
><end homolgy with PRLR_HUMAN, just N-terminal to transmembrane
domain in PRLR_HUMAN>
RSERPGPGGGACEPRGGEPSG PVRRELKQFLGWLKKHAYCS
><potential N-glycosylation site>
NLSFRLYDQWRAWMQSHKTRNQDEGILPSGRRGTARGPAR

FIGURE 18

CCCACGCGTCCGCTGGTGTAGATCGAGCAACCCTCTAAAAGCAGTTTAGAGTGGTAAAAAA
AAAAAAAAACACACCAAACGCTCGCAGCCACAAAAGGGATGAAATTTCTTCTGGACATCCTC
CTGCTTCTCCCGTTACTGATCGTCTGCTCCCTAGAGTCCTTCGTGAAGCTTTTTATTCTTAA
GAGGAGAAAATCAGTCACCGGCGAAATCGTGCTGATTACAGGAGCTGGGCATGGAATTGGGA
GACTGACTGCCTATGAATTTGCTAAACTTAAAAGCAAGCTGGTTCTCTGGGATATAAATAAG
CATGGACTGGAGGAAACAGCTGCCAAATGCAAGGGACTGGGTGCCAAGGTTTACATACCTTTGT
GGTAGACTGCAGCAACCGAGAAGATATTTACAGCTCTGCAAAGAAGGTGAAGGCAGAAATTG
GAGATGTTAGTATTTTAGTAAATAATGCTGGTGTAGTCTATACATCAGATTTGTTTGCTACA
CAAGATCCTCAGATTGAAAAGACTTTTGAAGTTAATGTACTTGACATTTCTGGACTACAAA
GGCATTTCCTTCTGCAATGACGAAGAATAACCATGGCCATATTGTCAGTGTGGCTTCGGCAG
CTGGACATGTCTCGGTCCCTTCTTACTGGCTTACTGTTCAAGCAAGTTTGCTGCTGTTGGA
TTTCATAAACTTTGACAGATGAACTGGCTGCCTTACAAATAACTGGAGTCAAACAACATG
TCTGTGTCCTAATTTTCGTAAACACTGGCTTCATCAAAAATCCAAGTACAAGTTTGGGACCCA
CTCTGGAACCTGAGGAAGTGGTAAACAGGCTGATGCATGGGATTCTGACTGAGCAGAAGATG
ATTTTTATTCCATCTTCTATAGCTTTTTTAACAACATTGGAAAGGATCCTTCCTGAGCGTTT
CCTGGCAGTTTTTAAACGAAAAATCAGTGTTAAGTTTGATGCAGTTATTGGATATAAAATGA
AAGCGCAATAAGCACCTAGTTTTCTGAAAACAGTTTACCAGGTTTAGGTTGATGTCATCTA
ATAGTGCCAGAATTTTAAATGTTTGAACCTCTGTTTTTTCTAATTATCCCCATTTCTTCAATA
TCATTTTTGAGGCTTTGGCAGTCTTCATTTACTACCACTTGTTCTTTAGCCAAAAGCTGATT
ACATATGATATAAACAGAGAAATACCTTTAGAGGTGACTTTAAGGAAAATGAAGAAAAAGAA
CCAAAATGACTTTATTAAATAATTTCCAAGATTATTTGTGGCTCACCTGAAGGCTTTGCAA
AATTTGTACCATAACCGTTTATTTAACATATATTTTTATTTTGGATTGCACTTAAATTTTGT
ATAATTTGTGTTTCTTTTTCTGTTCTACATAAAATCAGAACTTCAAGCTCTCTAAATAAAA
TGAAGGACTATATCTAGTGGTATTTTCACAATGAATATCATGAACTCTCAATGGGTAGGTTTC
ATCCTACCCATTGCCACTCTGTTTCCTGAGAGATACCTCACATTCCAATGCCAAACATTTCT
GCACAGGGAAGCTAGAGGTGGATACACGTGTTGCAAGTATAAAAGCATCACTGGGATTTAAG
GAGAATTGAGAGAATGTACCCACAAATGGCAGCAATAATAAATGGATCACACTTAAAAAAA
AA
AA

FIGURE 19

><subunit 1 of 1, 300 aa, 1 stop

><MW: 32964, pI: 9.52

><signal peptide>

MKFLLDILLLLPLLIVCSL

><start mature protein>

ESFVKLFIPKRRKSVTGEIVLITGAGHGIGRLTAYEFAKLKSKLVLDINKHGLEETAACK

GLGAKVHTFVVDCSNREDIYSSAKKVKAIEGDVSILVNNAGVVYTSDFATQDPQIEKTFEV

NVLAHFWTTKAFLPAMTKNNHGHIVTVASAAGHVSVPFLLA

><putative oxidoreductase active site, by similarity to
Y00P_MYCTU and BUDC_KLETE>

YCSSLFAAVGFHKTLTDELAALQITGVKTTCLCPNFVNTGFIKNPSTSLGPTLEPEEVNRL

MHGILTEQKMIFIPSSIAFLTTLERILPERFLAVLKRKISVKFDAVIGYKMQ

FIGURE 20

GACTAGTTCTCTTGGAGTCTGGGAGGAGGAAAGCGGAGCCGGCAGGGAGCGAACCAGGACTG
GGGTGACGGCAGGGCAGGGGGCGCCTGGCCGGGGAGAAGCGCGGGGGCTGGAGCACCACCAA
CTGGAGGGTCCGGAGTAGCGAGCGCCCCGAAGGAGGCCATCGGGGAGCCGGGAGGGGGGACT
GCGAGAGGACCCCGGCGTCCGGGCTCCCGGTGCCAGCGCTATGAGGCCACTCCTCGTCCTGC
TGCTCCTGGGCCTGGCGGGCCGGCTCGCCCCCACTGGACGACAACAAGATCCCCAGCCTCTGC
CCGGGGCACCCCGGCCTTCCAGGCACGCCGGGCCACCATGGCAGCCAGGGCTTGCCGGGGCCG
CGATGGCCGCGACGGCCGCGACGGCGCGCCCCGGGGCTCCGGGAGAGAAAGCGAGGGCGGGA
GGCCGGGACTGCCGGGACCTCGAGGGGACCCGGGCCGCGAGGAGAGGCGGGACCCGCGGGG
CCCACCGGGCCTGCCGGGGAGTGCTCGGTGCCTCCGCGATCCGCCTTCAGCGCCAAGCGCTC
CGAGAGCCGGGTGCCTCCGCCGTCTGACGCACCCTTGCCCTTCGACCGCGTGCTGGTGAACG
AGCAGGGACATTACGACGCCGTCACCGGCAAGTTCACCTGCCAGGTGCCTGGGGTCTACTAC
TTCGCCGTCCATGCCACCGTCTACCGGGCCAGCCTGCAGTTTGATCTGGTGAAGAATGGCGA
ATCCATTGCCTCTTTCTTCCAGTTTTTCGGGGGGTGGCCCAAGCCAGCCTCGCTCTCGGGGG
GGCCATGGTGAGGCTGGAGCCTGAGGACCAAGTGTGGGTGCAGGTGGGTGTGGGTGACTAC
ATTGGCATCTATGCCAGCATCAAGACAGACAGCACCTTCTCCGATTTCTGGTGTACTCCGA
CTGGCACAGCTCCCCAGTCTTTGCTTAGTGCCCACTGCAAAGTGAGCTCATGCTCTCACTCC
TAGAAGGAGGGTGTGAGGCTGACAACCAGGTCATCCAGGAGGGCTGGCCCCCTGGAATATT
GTGAATGACTAGGGAGGTGGGGTAGAGCACTCTCCGTCTGCTGCTGGCAAGGAATGGGAAC
AGTGGCTGTCTGCGATCAGGTCTGGCAGCATGGGGCAGTGGCTGGATTTCTGCCCAAGACCA
GAGGAGTGTGCTGTGCTGGCAAGTGTAAGTCCCCCAGTTGCTCTGGTCCAGGAGCCACGGT
GGGGTGCTCTCTTCTGCTGCTCTGCTTCTCTGGATCCTCCCCACCCCTCCTGCTCCTGGG
GCCGGCCCTTTCTCAGAGATCACTCAATAAACCTAAGAACCCTCATAAAAAAAAAAAAAAA
AAAAAAAAAAAAA

FIGURE 21

><subunit 1 of 1, 243 aa, 1 stop

><MW: 25298, pI: 6.44, NX(S/T): 0

<signal peptide>

MRPLLVLALLGLAAG

<start of mature protein>

SPPLDDNKIPSLCPGHPGLPGTPGHHGSQGLPGRDGRDGRDGAPGAPGEKGE

<potential N-myristolation site>

GGRPGLPGPRGDPGPRGEAGPAGPTGPAGECSVPPRSAFSKRSESRVPPPSDAPLPFDRVL

VNEQGHYDAVTGKFTCQVPGVYYFAVHATVYRASLQFDLVKNGESIASFFQFFGGWPKPASL

SGGAMVRLEPEDQVWVQVGVDYI

<potential N-myristolation site>

GIYASIKTDSTFSGFLVYSDWHSSPVFA

FIGURE 22

CTCTTTTGTCCACCAGCCCAGCCTGACTCCTGGAGATTGTGAATAGCTCCATCCAGCCTGAG
AAACAAGCCGGGTGGCTGAGCCAGGCTGTGCAEGGAGCACCTGACGGGCCCAACAGACCCAT
GCTGCATCCAGAGACCTCCCCTGGCCGGGGGCATCTCCTGGCTGTGCTCCTGGCCCTCCTTG
GCACCACCTGGGCAGAGGTGTGGCCACCCAGCTGCAGGAGCAGGCTCCGATGGCCGGAGCC
CTGAACAGGAAGGAGAGTTTCTTGCTCCTCTCCCTGCACAACCGCCTGCGCAGCTGGGTCCA
GCCCCCTGCGGCTGACATGCGGAGGCTGGACTGGAGTGACAGCCTGGCCCAACTGGCTCAAG
CCAGGGCAGCCCTCTGTGGAATCCCAACCCCGAGCCTGGCATCCGGCCTGTGGCGCACCCCTG
CAAGTGGGCTGGAACATGCAGCTGCTGCCCGCGGGCTTGGCGTCCTTTGTTGAAGTGGTCAG
CCTATGGTTTGCAGAGGGGCAGCGGTACAGCCACGCGGCAGGAGAGTGTGCTCGCAACGCCA
CCTGCACCCACTACACGCAGCTCGTGTGGGCCACCTCAAGCCAGCTGGGCTGTGGGCGGCAC
CTGTGCTCTGCAGGCCAGACAGCGATAGAAGCCTTTGTCTGTGCCTACTCCCCCGGAGGCAA
CTGGGAGGTCAACGGGAAGACAATCATCCCCATAAGAAGGGTGCCTGGTGTTTCGCTCTGCA
CAGCCAGTGTCTCAGGCTGCTTCAAAGCCTGGGACCATGCAGGGGGGCTCTGTGAGGTCCCC
AGGAATCCTTGTGCGATGAGCTGCCAGAACCATGGACGTCTAACATCAGCACCTGCCACTG
CCACTGTCCCCCTGGCTACACGGGCAGATACTGCCAAGTGAGGTGCAGCCTGCAGTGTGTGC
ACGGCCGGTTCCGGGAGGAGGAGTGCTCGTGCGTCTGTGACATCGGCTACGGGGGAGCCCAG
TGTGCCACCAAGGTGCATTTTCCCTTCCACACCTGTGACCTGAGGATCGACGGAGACTGCTT
CATGGTGCTTTCAGAGGCAGACACCTATTACAGAGCCAGGATGAAATGTCAGAGGAAAGGCG
GGGTGCTGGCCAGATCAAGAGCCAGAAAGTGAGGACATCCTCGCCTTCTATCTGGGCCGC
CTGGAGACCACCAACGAGGTGACTGACAGTGACTTCGAGACCAGGAACTTCTGGATCGGGCT
CACCTACAAGACCGCCAAGGACTCCTTCCGCTGGGCCACAGGGGAGCACCAGGCCTTCACCA
GTTTTGCCTTTGGGCAGCCTGACAACCACGGGCTGGTGTGGCTGAGTGTGCCATGGGGTTT
GGCAACTGCGTGAGCTGCAGGCTTCAGCTGCCTTCAACTGGAACGACCAGCGCTGCAAAAC
CCGAAACCGTTACATCTGCCAGTTTGCCAGGAGCACATCTCCCGGTGGGGCCCAGGGTCCT
GAGGCCTGACCACATGGCTCCCTCGCCTGCCCTGGGAGCACCGGCTCTGCTTACCTGTCTGC
CCACCTGTCTGGAACAAGGGCCAGGTTAAGACCACATGCCTCATGTCCAAAGAGGTCTCAGA
CCTTGCACAATGCCAGAAGTTGGGCAGAGAGAGGCAGGGAGGCCAGTGAGGGCCAGGGAGTG
AGTGTTAGAAGAAGCTGGGGCCCTTCGCCTGCTTTTGATTGGGAAGATGGGCTTCAATTAGA
TGGCGAAGGAGAGGACACCGCCAGTGGTCCAAAAGGCTGCTCTCTTCCACCTGGCCCAGAC
CCTGTGGGGCAGCGGAGCTTCCCTGTGGCATGAACCCACGGGGTATTAAATTATGAATCAG
CTGAAAAAAAAAAAAA

FIGURE 23

><homology to cysteine-rich secretory proteins>

><signal peptide>

MLHPETSPGRGHLLAVLLALLGTTWA

><start mature protein>

EVWPPQLQEQAPMAGALNRKESFLLLSLHNRLRSWVQPPAADMRRLDWSDSLAQLAQARAAL

CGIPTPSLASGLWRTLQVGWNMQLLPAGLASFVEVSLWFAEGQRYSHAAGECAR

><potential N-glycosylation site>

NATCTHYTQLVWATSSQLGCGRHLCSAGQTAIEAFVCLAYSPGGNWEVNGKTIIPYKKGAWCS

LCTASVSGCFKAWDHAGGLCEVPRNPCRMSCQNHGRL

><potential N-glycosylation site>

NISTCH

><EGF-like domain cysteine pattern signature>

CHCPPGYTGRYCQVRCSLQCVHGRFREEECS

><EGF-like domain cysteine pattern signature>

CVCDIGYGGAQCATKVHFPFHTCDLRIDGDCFMVSSEADTYRARMKCQRKGGVLAQIKSQK

VQDILAFYLGRLETTNEVTDSDFETRNFWIGLTYKTAKDSFRWATGEHQAFTSFAFGQPDNH

GLVWLSAAMGFGN

><C-type lectin domain signature (CVELQASAAFNWNDQRCKTRNRYIC)>

CVELQASAAFNWNDQRCKTRNRYICQFAQEHISRWGPGS

FIGURE 24

CGGACGCGTGGGCTGGGCGCTGCAAAGCGTGTCCCGCCGGGTCCCCGAGCGTCCCGCGCCCT
CGCCCCGCCATGCTCCTGCTGCTGGGGCTGTGCCTGGGGCTGTCCCTGTGTGTGGGGTCGCA
GGAAGAGGCGCAGAGCTGGGGCCACTCTTCGGAGCAGGATGGACTCAGGGTCCCCGAGGCAAG
TCAGACTGTTGCAGAGGCTGAAAACCAAACCTTTGATGACAGAATTCTCAGTGAAGTCTACC
ATCATTTCCCGTTATGCCTTCACTACGGTTTCTGCAGAATGCTGAACAGAGCTTCTGAAGA
CCAGGACATTGAGTTCAGATGCAGATTCCAGCTGCAGCTTTCATCACCAACTTCACTATGC
TTATTGGAGACAAGGTGTATCAGGGCGAAATTACAGAGAGAGAAAAGAAGAGTGGTGATAGG
GTAAAAGAGAAAAGGAATAAAACCACAGAAGAAAATGGAGAGAAGGGGACTGAAATATTAG
AGCTTCTGCAGTGATTCCCAGCAAGGACAAAGCCGCCTTTTTCCTGAGTTATGAGGAGCTTC
TGCAGAGGCGCCTGGGCAAGTACGAGCACAGCATCAGCGTGCGGCCCCAGCAGCTGTCCGGG
AGGCTGAGCGTGGACGTGAATATCCTGGAGAGCGCGGGCATCGCATCCCTGGAGGTGCTGCC
GCTTCACAACAGCAGGCAGAGGGGCGAGTGGGCGCGGGGAAGATGATTCTGGGCCTCCCCCAT
CTACTGTCATTAACCAAAATGAAACATTTGCCAACATAATTTTAAACCTACTGTAGTACAA
CAAGCCAGGATTGCCCAGAATGGAATTTTGGGAGACTTTATCATTAGATATGACGTCAATAG
AGAACAGAGCATTGGGGACATCCAGGTTCTAAATGGCTATTTTGTGCACTACTTTGCTCCTA
AAGACCTTCTCCTTTACCCAAGAATGTGGTATTCGTGCTTGACAGCAGTGCTTCTATGGTG
GGAACCAAACTCCGGCAGACCAAGGATGCCCTCTTACAATTCTCCATGACCTCCGACCCCA
GGACCGTTTTCAGTATCATTGGATTTTCCAACCGGATCAAAGTATGGAAGGACCACTTGATAT
CAGTCACTCCAGACAGCATCAGGGATGGGAAAGTGACATTACCATATGTCACCCACTGGA
GGCACAGACATCAACGGGGCCCTGCAGAGGGCCATCAGGCTCCTCAACAAGTACGTGGCCCA
CAGTGGCATTGGAGACCGGAGCGTGTCCCTCATCGTCTTCTGACGGATGGGAAGCCCACGG
TCGGGGAGACGCACACCCTCAAGATCCTCAACAACACCCGAGAGGCCGCCCGAGGCCAAGTC
TGCATCTTCACCATTGGCATCGGCAACGACGTGGACTTCAGGCTGCTGGAGAACTGTGCT
GGAGAACTGTGGCCTCACACGGCGCGTGCACGAGGAGGAGGACGCAGGCTCGCAGCTCATCG
GGTTCATCGATGAAATCAGGACCCCGCTCCTCTCTGACATCCGCATCGATTATCCCCCAGC
TCAGTGGTGCAGGCCACCAAGACCCTGTTCCCCAACTACTTCAACGGCTCGGAGATCATCAT
TGCGGGGAAGCTGGTGGACAGGAAGCTGGATCACCTGCACGTGGAGGTCACCGCCAGCAACA
GTAAGAAATTCATCATCCTGAAGACAGATGTGCCTGTGCGGCCTCAGAAGGCAGGGAAAGAT
GTCACAGGAAGCCCCAGGCCTGGAGGCGATGGAGAGGGGGACACCAACCACATCGAGCGTCT
CTGGAGCTACCTACCAACAAAGGAGCTGCTGAGCTCCTGGCTGCAAAGTGACGATGAACCGG
AGAAGGAGCGGCTGCGGCAGCGGGCCAGGCCCTGGCTGTGAGCTACCGCTTCTCACTCCC
TTCACCTCCATGAAGCTGAGGGGGCCGTCACACGCATGGATGGCCTGGAGGAGGCCACGG
CATGTGCGCTGCCATGGGACCCGAACCGGTGGTGCAGAGCGTGCGAGGAGCTGGCACGCAGC
CAGGACCTTTGCTCAAGAAGCCAACTCCGTCAAAAAAAAAACAAAACAAAACAAAAAAGA
CATGGGAGAGATGGTGTTTTTCCTCTCCACCACCTGGGGATACGATGAGAAGATGGCCACCT
GCAAGCCAGGAAGACGGCCTCACCAGACACCATGTCTGCTGGCACCTTGATCTTGACCTC
CCAGCCTCCAGAACTGTGAGAAATAAATGTGTTTTGTTTAAGCTAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIGURE 25

><homology to inter-alpha-trypsin inhibitor heavy chain-related proteins>

><signal peptide>

MLLLLGLCLGLSLC

><start mature protein>

VGSQEEAQSWGHSSEQDGLRVPRQVRLQLKTKPLMTEFSVKSTIISRYAFTTVSCRMLNR
ASEDQDIEFQMQIPAAAFIT

><potential N-glycosylation site>

NFTMLIGDKVYQGEITEREKKSGDRVKEKR

><potential N-glycosylation site>

NKTTEENGEKGTEIFRASAVIPSKDKAAFFLSYEELLQRRLGKYEHSISVRPQQLSGRLSVD
VNILESAGIASLEVLPLHNSRQRGSGRGEDDSGPPPSTVINQ

><potential N-glycosylation site>

NETFANIIIFKPTVVQQARIAQNGILGDFIIRYDVNREQSIGDIQVLNGYFVHYFAPKDLPL
PKNVVFLDSSASMVGTKLRQTKDALFTILHDLRPQDRFSIIGFSNRIKVKDHLISVTPDS
IRDGKVYIHHMSPTGGTDINGALQRAIRLLNKYVAHSGIGDRSVSLIVFLTDGKPTVGETHT
LKIL

><potential N-glycosylation site>

NNTREAARGQVCIFTIGINDVDFRLLEKLSLENCGLTRRVHEEEDAGSQLIGFYDEIRTPL
LSDIRIDYPPSSVVQATKTLFPNYF

><potential N-glycosylation site>

NGSEIIIAGKLVDRKLDHLHVEVTASNSKKFIILKTDVPVRPQKAGKDVGTGSPRPGDGEGD
TNHIERLWSYLTTKELLSSWLQSDDEPEKERLRQRAQALAVSYRFLTPFTSMKLRGPVPRMD
GLEEAHGMSAAMGPEPVVQSVRGAGTQPGPLLKKPNSVKKKQ

><potential N-glycosylation site>

NKTKKRHGRDGVFPLHHLGIR

FIGURE 26

CGGACGCGTGGGGTGCCCGACATGGCGAGTGTAGTGCTGCCGAGCGGATCCCAGTGTGCGGC
GGCAGCGGCGGCGGCGGCGCCTCCCGGGCTCCGGCTTCTGCTGTTGCTCTTCTCCGCCGCGG
CACTGATCCCCACAGGTGATGGGCAGAATCTGTTTACGAAAGACGTGACAGTGTATCGAGGGA
GAGGTTGCGACCATCAGTTGCCAAGTCAATAAGAGTGACGACTCTGTGATTCTAGCTACTGAA
TCCCAACAGGCAGACCATTATTTTTCAGGGACTTCAGGCCTTTGAAGGACAGCAGGTTTCAGT
TGCTGAATTTTTCTAGCAGTGAACCTCAAAGTATCATTGACAAACGTCTCAATTTCTGATGAA
GGAAGATACTTTTGCCAGCTCTATACCGATCCCCACAGGAAAGTTACACCACCATCACAGT
CCTGGTCCCACCACGTAATCTGATGATCGATATCCAGAAAGACACTGCGGTGGAAGGTGAGG
AGATTGAAGTCAACTGCACTGCTATGGCCAGCAAGCCAGCCACGACTATCAGGTGGTTCAAA
GGGAACACAGAGCTAAAAGGCAAATCGGAGGTGGAAGAGTGGTCAGACATGTACACTGTGAC
CAGTCAGCTGATGCTGAAGGTGCACAAGGAGGACGATGGGGTCCCAGTGATCTGCCAGGTGG
AGCACCTGCGGTCACTGGAAACCTGCAGACCCAGCGGTATCTAGAAGTACAGTATAAGCCT
CAAGTGCACATTCAGATGACTTATCCTCTACAAGGCTTAACCCGGGAAGGGGACGCGCTTGA
GTTAACATGTGAAGCCATCGGGAAGCCCCAGCCTGTGATGGTAACTTGGGTGAGAGTCGATG
ATGAAATGCCTCAACACGCCGTAAGTGTCTGGGCCCAACCTGTTTCATCAATAACCTAAACAAA
ACAGATAATGGTACATACCGCTGTGAAGCTTCAAACATAGTGGGGAAAGCTCACTCGGATTA
TATGCTGTATGTATACGATCCCCCACAACCTATCCCTCCTCCCACAACAACCACCACCACCA
CCACCACCACCACCACCACCATCCTTACCATCATCACAGATTCCCGAGCAGGTGAAGAAGGC
TCGATCAGGGCAGTGGATCATGCCGTGATCGGTGGCGTCGTGGCGGTGGTGGTGTTCGCCAT
GCTGTGCTTGCTCATCATTCTGGGGCGCTATTTTGCCAGACATAAAGGTACATACTTCACTC
ATGAAGCCAAAGGAGCCGATGACGCAGCAGACGCAGACACAGCTATAATCAATGCAGAAGGA
GGACAGAACAACCTCCGAAGAAAAGAAAGAGTACTTCATCTAGATCAGCCTTTTTGTTTCAAT
GAGGTGTCCAACCTGGCCCTATTTAGATGATAAAGAGACAGTGATATTGG

FIGURE 27

><signal peptide>
MASVVLPSGSQCAAAAAAAPPGLRLLLLLFSAAAL
><start mature protein>
IPTGDGQNLFTKDVTVIEGEVATI
><Ig repeats in extracellular domain>
SCQV
><potential N-glycosylation site>
NKSDDSVIQLLNPNRQTIYFRDFRPLKDSRFQLL
><potential N-glycosylation site>
NFSSELKVSLT
><potential N-glycosylation site>
NVSISDEGRYFCQLYTDPPQESYTTITVLVPPRNLMDIQKDTAVEGEEIEV
><potential N-glycosylation site>
NCTAMASKPATTIRWFKGNTELKGKSEVEEWSMYTVTSQMLKVHKEDDGVPVICQVEHPA
VTGNLQTQRYLEVQYKPQVHIQMTYPLQGLTREGDALELTCEAIGKPQPMVTWVRVDDEMP
QHAVLSGPNLFINNL
><potential N-glycosylation site>
NKTD
><potential N-glycosylation site>
NGTYRCEASNIVGKAHSDYMLYVYDPPTTIPPTTTTTTTTTTTTTTILTIITDSRAGEEG
SIRAVDH
><potential transmembrane domain>
AVIGGVVAVVVFAMLCLLIIL
><end potential transmembrane domain>
GRYFARHKGTYFTHEAKGADDAADADTAIINAEGGQNNSEEKKEYFI

FIGURE 28

GGGGCGGGTGGACGCGGACTCGAACGCAGTTGCTTCGGGACCCAGGACCCCTCGGGCCCGA
CCCGCCAGGAAAGACTGAGGCCGCGGCCTGCCCCGCGGCTCCCTGCGCGCGCGCGGCTC
CCGGGACAGAAGATGTGCTCCAGGGTCCCTCTGCTGCTGCCGCTGCTCCTGCTACTGGCCCT
GGGGCCTGGGGTGCAGGGCTGCCCATCCGGCTGCCAGTGCAGCCAGCCACAGACAGTCTTCT
GCACTGCCCGCCAGGGGACCACGGTGCCCCGAGACGTGCCACCCGACACGGTGGGGCTGTAC
GTCTTTGAGAACGGCATCACCATGCTCGACGCAAGCAGCTTTGCCGGCCTGCCGGGCTGCA
GCTCCTGGACCTGTACAGAACCAGATCGCCAGCCTGCGCCTGCCCCGCTGCTGCTGCTGG
ACCTCAGCCACAACAGCCTCCTGGCCCTGGAGCCCGGCATCCTGGACACTGCCAACGTGGAG
GCGCTGCGGCTGGCTGGTCTGGGGCTGCAGCAGCTGGACGAGGGGCTCTTCAGCCGCTTGCG
CAACCTCCACGACCTGGATGTGTCCGACAACCAGCTGGAGCGAGTGCCACCTGTGATCCGAG
GCCTCCGGGGCCTGACGCGCCTGCGGCTGGCCGGCAACACCCGCATTGCCAGCTGCGGCCC
GAGGACCTGGCCGGCCTGGCTGCCCTGCAGGAGCTGGATGTGAGCAACCTAAGCCTGCAGGC
CCTGCCTGGCGACCTCTCGGGCCTCTTCCCCGCTGCGGCTGCTGGCAGCTGCCCGCAACC
CCTTCAACTGCGTGTGCCCCCTGAGCTGGTTTGGCCCTGGGTGCGCGAGAGCCACGTCACA
CTGGCCAGCCCTGAGGAGACGCGCTGCCACTTCCCGCCCAAGAACGCTGGCCGGCTGCTCCT
GGAGCTTGACTACGCCGACTTTGGCTGCCAGCCACCACCACACAGCCACAGTGCCCAACA
CGAGGCCCCGTGGTGCGGGAGCCACAGCCTTGCTTCTAGCTTGGCTCCTACCTGGCTTAGC
CCCACAGCGCCGGCCACTGAGGCCCCAGCCCGCCCTCCACTGCCCCACCGACTGTAGGGCC
TGTCCCCCAGCCCCAGGACTGCCACCGTCCACCTGCCTCAATGGGGGCACATGCCACCTGG
GGACACGGCACCACCTGGCGTGCTTGTCGCCCGAAGGCTTCACGGGCCTGTACTGTGAGAGC
CAGATGGGGCAGGGGACACGGCCCAGCCCTACACCAGTCACGCCGAGGCCACCACGGTCCCT
GACCTTGGGCATCGAGCCGGTGAGCCCCACCTCCCTGCGCGTGCGGCTGCAGCGCTACCTCC
AGGGGAGCTCCGTGCAGCTCAGGAGCCTCCGTCTCACCTATCGCAACCTATCGGGCCCTGAT
AAGCGGCTGGTGACGCTGCGACTGCCTGCCTCGCTCGCTGAGTACACGGTCACCCAGCTGCG
GCCCAAGCCACTTACTCCGTCTGTGTATGCCTTTGGGGCCCGGGCGGGTGCCGGAGGGCG
AGGAGGCCTGCGGGGAGGGCCATACACCCCAAGCCGTCCACTCCAACCACGCCCCAGTCACC
CAGGCCCCGAGGGCAACCTGCCGCTCCTCATTGCGCCCGCCCTGGCCGCGGTGCTCCTGGC
CGCGCTGGCTGCGGTGGGGGCAGCCTACTGTGTGCGGCGGGGGCGGGCCATGGCAGCAGCGG
CTCAGGACAAAGGGCAGGTGGGGCCAGGGGCTGGGCCCCCTGGAACCTGGAGGGAGTGAAGGTC
CCCTTGAGGCCAGGCCCCGAAGGCAACAGAGGGCGGTGGAGAGGCCCTGCCAGCGGGTCTGA
GTGTGAGGTGCCACTCATGGGCTTCCCAGGGCCTGGCCTCCAGTCACCCCTCCACGCAAGC
CCTACATCTAAGCCAGAGAGAGACAGGGCAGCTGGGGCCGGGCTCTCAGCCAGTGAGATGGC
CAGCCCCCTCCTGCTGCCACACCACGTAAGTTCTCAGTCCCAACCTCGGGGATGTGTGCAGA
CAGGGCTGTGTGACCACAGCTGGGGCCTGTTCCCTCTGGACCTCGGTCTCCTCATCTGTGAG
ATGCTGTGGCCAGCTGACGAGCCCTAACGTCCCCAGAACCGAGTGCCTATGAGGACAGTGT
CCGCCCTGCCCTCCGCAACGTGCAGTCCCTGGGCACGGCGGGCCCTGCCATGTGCTGGTAAC
GCATGCCTGGGCCCTGCTGGGCTCTCCCACTCCAGGCGGACCCTGGGGGCCAGTGAAGGAAG
CTCCCGGAAAGAGCAGAGGGAGAGCGGGTAGGCGGCTGTGTGACTCTAGTCTTGGCCCCAGG
AAGCGAAGGAACAAAAGAACTGGAAAGGAAGATGCTTTAGGAACATGTTTTGCTTTTTTAA
AATATATATATATTTATAAGAGATCCTTTCCCATTTATTCTGGGAAGATGTTTTTCAAACCTC
AGAGACAAGGACTTTGGTTTTTTGTAAGACAAACGATGATATGAAGGCCTTTTGTAAGAAAAA
ATAAAAAAAAAA

FIGURE 29

><signal peptide>

MCSRVPLLLLPLLLLLLALGPGVQ

><start mature protein>

G

><homology to ALS_HUMAN and other leucine-repeat rich proteins
in extracellular domain>

CPSGCQCSQPQTVFCTARQGTTPRDVPPDTVGLYVFENGITMLDASSFAGLPGLQLLDLSQ
NQIASLRLPRLLLLDLSHNSLLALEPGILDANVEALRLAGLGLQQLDDEGLFSRLRNLDLD
VSDNQLERVPPVIRGLRGLTRLRLAGNTRIAQLRPEDLAGLAALQELDVS

><potential N-glycosylation site>

NLSLQALPGDLSGLFPRLRLAAARNPFNCVCPWSWFGPWVRESHVTLASPEETRCHFPPKN
AGRLLLELDYADFGCPATTTTATVPTTRPVVREPTALSSSLAPTWLSPTAPATEAPSPPSTA
PPTVGVPVQPQDCPPSTCLNGGTCHLGTRHHLA

><EGF-like domain cysteine pattern signature>

CLCPEGFTGLYCESQMGQGTRPSPTPVTPRPPRSLTLGIEPVSPSLRVGLQRYLQGSSVQL
RSLRLTYR

><potential N-glycosylation site>

NLSGPDKRLVTLRLPASLAEYTVTQLRP

><potential N-glycosylation site>

NATYSVCMPLGPGRVPEGEEACGEAHTPPAVHSNHAPVTQAREGNLPLLIAP

><potential transmembrane domain>

ALAAVLLAALAAGVGAAYCV

><end transmembrane domain>

RRGRAMAAAAQDKGQVGPGAGPLELEGVKVPLEPGPKATEGGGEALPSGSECEVPLMGFP
GLQSPLHAKPYI

FIGURE 30

GGCACTAGGACAACCTTCTTCCCTTCTGCACCACTGCCCCGTACCCTTACCCGCCCCGCCACC
TCCTTGCTACCCCACTCTTGAAACCACAGCTGTTGGCAGGGTCCCCAGCTCATGCCAGCCTC
ATCTCCTTTCTTGCTAGCCCCCAAAGGGCCTCCAGGCAACATGGGGGGGCCAGTCAGAGAGC
CGGCACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGGCAGCTCTGGGGGGCCGTGGCTTGTCGC
ATGGCTCTGCTGACCCAACAAACAGAGCTGCAGAGCCTCAGGAGAGAGGTGAGCCGGCTGCA
GGGGACAGGAGGCCCCCTCCCAGAATGGGGAAGGGTATCCCTGGCAGAGTCTCCCGGAGCAGA
GTTCCGATGCCCTGGAAGCCTGGGAGAATGGGGAGAGATCCCGGAAAAGGAGAGCAGTGCTC
ACCCAAAAACAGAAGAAGCAGCACTCTGTCCTGCACCTGGTTCCCATTAACGCCACCTCCAA
GGATGACTCCGATGTGACAGAGGTGATGTGGCAACCAGCTCTTAGGCGTGGGAGAGGCCTAC
AGGCCCCAAGGATATGGTGTCCGAATCCAGGATGCTGGAGTTTATCTGCTGTATAGCCAGGTC
CTGTTTCAAGACGTGACTTTCACCATGGGTCAGGTGGTGTCTCGAGAAGGCCAAGGAAGGCA
GGAGACTCTATTCCGATGTATAAGAAGTATGCCCTCCCACCCGGACCGGGCCTACAACAGCT
GCTATAGCGCAGGTGTCTTCCATTTACACCAAGGGGATATTCTGAGTGTGATAATTCCCCGG
GCAAGGGCGAAACTTAACCTCTCTCCACATGGAACCTTCCTGGGGTTTGTGAAACTGTGATT
GTGTTATAAAAAGTGGCTCCCAGCTTGGAAGACCAGGGTGGGTACATACTGGAGACAGCCAA
GAGCTGAGTATATAAAGGAGAGGGAATGTGCAGGAACAGAGGCATCTTCCTGGGTTTGGCTC
CCCGTTCCTCACTTTTCCCTTTTCATTCCCACCCCTAGACTTTGATTTTACGGATATCTTG
CTTCTGTTCCCCATGGAGCTCCG

FIGURE 31

<MW: 27433, pI: 9.85, NX(S/T): 2

MPASSPFLAPKGPPGNMGGPVREPALSVALWSGAALGAVACAMALLTQOTELQSLRREV
SRLQGTGGPSQNGEGYPWQSLPEQSSDALEAWENGERSRKRRAVLTQKQKKQHSVLHLVPIN
ATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREG
QGRQETLFR CIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVII PRARAKLNLS PHGTFLGFVKL

FIGURE 32

DNA52722 1 MPASSPFL LAPKGPPGNMGGPVREPALSVALWLSWGAALGAVACAMALIT
TNFA_HUMAN 1MSTESMIRDVELAEEALP

DNA52722 51 QQTTELQSLRREVSRLQGTGGPSONSEGYPWQSLPEQSSDALEAWENGERS
TNFA_HUMAN 19 KKTGGPOGSRCLFLSLFSFLIVAGATTFLCLLHFGVIGP--QREEFPRD

DNA52722 101 RKRRAVLTQKQKKOHSVLHLVPINATSKDDSDVTEVMWOPALRRGRGLQA
TNFA_HUMAN 67 LSLISPLAQAVRSSSRTPSDKPVAHVVANPQAEGLQWLN--RRANALLA

DNA52722 151 QGYGVR-----IQDAGVYLLYSQVLFQD-----VTFTMGQVVSRREG---D
TNFA_HUMAN 115 NGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSHTVLLTHTISRIVASYD

DNA52722 188 GRQETL----FRCI RSMPSHPDRA--YNSCY SAGVFHLHOGDPLSVIIPR
TNFA_HUMAN 165 TKVNLLSAIKSPQRETPEGAEAKPWYEPYLGGVFQLEKGDRLSAEINR

DNA52722 232 ARAKLNLSPHG-TFLGFVKL
TNFA_HUMAN 215 P-DYLDFAESQVYFGIIAL

FIGURE 33

h-CD27L 1MPEEGSGCSVRRRPYGCVLRAALVPLVAG.....
h-CD30L 1MDPGLQOALNGMAPPDGTAMHVPAGSVASHLGTTSRSFYLT.....
h-CD40L 1 MIETYNQTS[PS]RSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIE.....
h-TNF 1MSTESMIRDVELAEALPKKTGGPQG.....
h-TRAIL 1 MAMMEVQGG[PS]..LGOTCVLIVIFTVLLQSLCAVAVTVVYFTNELKQMDDKYSKSG.....
h-TRANCE 1QMDP.....NRIS..EDGTHCIYRILRLHENADFODTTLESO.....
h-FASL 1 MQQPFNYPP[PO]IYWVDSSASSPWAPP[GT]VLP[CP]TSVPRRP[GP]RRPPPPPPPPPLPPPPPP
h-OX40L 1MERVQPLEENVGNAARPRFER.....
DNA52722 1 ----MPASS[PF]LLAPKGP[PG]NMGGPVREP[AL]SVALWLSWGAALGAVACAMALLT.....

h-CD27L 30LVIC[Q]VVCIQRFADQAOOLPLES[Q]GWDVAELQLNHTGPQODP
h-CD30L 43TATLALCLVFTVATIMVLVVORTDSIPNSPDNVPLKGGNCSE
h-CD40L 55DERNLHEDFVFMKTIQRCNTGERS[PS]LLNCEEIKSQFEGFVKD
h-TNF 27SRRC[Q]FLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRLS
h-TRAIL 54 --IACFLKEDDSYWDPNDEESMNSPCWQVKWQLROLVRKMILRTSEETISTVOEKQONIS
h-TRANCE 36DTKLIPDSCRRIKQAFQGA[VQ]KELQHVGSQHIRAEKAMVDGS
h-FASL 61 PPLPPLPLPPLKRGNHSTGLC[Q]LVMFFMVLVVALVGLGLGMFOLFHLQKELAE[RE]STSQ
h-OX40L 22NKLLLVASV[IQ]GLGLLLCFTYIC[Q]HFSALQVSHRYPRIOSIK
DNA52722 51QOTEL[Q]SLRREVSRLQGTGGPSQNGEGYPWQSLPEQSSDALE

h-CD27L 72 RLYWQGGPALGRSFLHGPELD.....-KGQ
h-CD30L 85 DLLCILKRAPFKKSWAYLQVAKHLNKT[KL]SWN.....-KDGILHGVRYQD
h-CD40L 98 IMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLN
h-TNF 69 LISPLAQAVRSSRTPSOKPVAHVANP.....-QAEGQLQWLNNRRANALLANGVELRD
h-TRAIL 112PLVRE[RG]PQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRN
h-TRANCE 79 WLDLAKRSKLEAQPFAHLTINATDIPSG.....-SHKVSLS[SW]YHDR-GWGKISNMTFSN
h-FASL 121MHTASSLEKQIGHPSPPPEKKELRKVAHLTG--KSNRSRSMPL[EW]EDTYGIVLLSGVKYKK
h-OX40L 64 VQFTEYKKEKGFILTSOKED.....-EIMKVON
DNA52722 93 AWENGERSRKRAVLTQKQKKQHSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQG

h-CD27L 96 LR--[I]HRD[Q]IYMVHI[QV]TLAICSSTTASRHHP.....-TTLAVGICSPASRS
h-CD30L 129GN-[LV]IQFPGLYF[FI]CQLQFLYQCPNNSVDLK.....-LELLINKHIKKOALVTV
h-CD40L 158GKOLTVKROGLYYIYAQVTFC[SN]REASSQAP.....-FIASLCLKSPGRFERILLRAAN
h-TNF 122NQ-[LV]VPSEGLYLIYSQVLFKGGGCPSTHVLLTHTISRIAVSYOTKVNLLSAIKSPCORE
h-TRAIL 172GE-[LV]THEKGFYYLYSOTYFRFOEEIKENTKNDKQMVQYIYKYTS-YPDPILLMK[SAR]NS
h-TRANCE 132GK-[LV]INQDGFYYLYANICFRHHETSGDLATEYLQLMVYVTKTSIKIPSSHTLMKGGSTK
h-FASL 179GG-[LV]INETGLYFVYSK[VY]FRGQSCNNLPLS.....-HKVYMRNSKYPQDLVMMEGKMMS
h-OX40L 91 NS-[VI]INC[DG]FYLL[TS]LKGYSFQEVN[IS]LHYQ.....-K-DEEPLFQLKKVRSV
DNA52722 153YG-VRIQDAGVYLLYSQVLE[Q]ODVTFTMGQVVS.....-REGOGROETLFRCI

h-CD27L 140ISLLR[LS]FHOGCTIVSQRLT[PL]AR[QD]TLCTNLTGTL[PS]RN-TDETF[FG]VQWVRP...
h-CD30L 177CESGMOTKHVYQNL[SO]FLLDYLOVNTTISVNVOTFOYIDTSTFPLENVLSIFLYSNSD
h-CD40L 211THSSAKPCGQQS-[IHL]GGVFELQ[PS]ASVFNVVTDPSQVSHG-TGF[TS]FGLLKL.....
h-TNF 181TPEGAEAKPWYEP[IL]YLGGVFQLEK[GD]RLSAEINRPDYLDFAESGOVYFGI[IAL].....
h-TRAIL 230CWSKDAEYGLYS-[IY]QCGIFELKENDRIFMSVTNEH[IL]DMD-HEASFFGAFLVG...
h-TRANCE 191YWSGNSEFHYS-[IN]VG[GF]FKLRS[EE]ISIEVSNPSLLDPD-ODATYFGAFKVRDID-
h-FASL 232YCTTGQMWARSS-[YL]GAVENLTSADHLYVNVSELSLVNFE-ESQTF[FG]LYKL.....
h-OX40L 136NSLMVASLTYKDKVYLNVT[TD]NTSLD[DF]HVN[GG]ELI[IL]HQN--PGE[FG]CVL.....
DNA52722 198RSMPSHPDRAVNSCYSA[QV]FHLHOGDILSVIIPRAKLNLSPHGTF[FG]FVKL.....

FIGURE 34

CACTTTCTCCCTCTCTTCTTTACTTTTCGAGAAACCGCGCTTCCGCTTCTGGTCGCAGAGAC
CTCGGAGACCGCGCCGGGGAGACGGAGGTGCTGTGGGTGGGGGGACCTGTGGCTGCTEGTA
CCGCCCCCACCCTCCTCTTCTGCACTGCCGTCTCCGGAAGACCTTTTCCCCTGCTCTGTT
TCCTTCACCGAGTCTGTGCATCGCCCCGACCTGGCCGGGAGGAGGCTTGGCCGGCGGGAGA
TGCTCTAGGGGCGGCGGGAGGAGCGGCCGGCGGGACGGAGGGCCCGGCAGGAAGATGGGC
TCCCGTGGACAGGGACTCTTGCTGGCGTACTGCCTGCTCCTTGCTTTGCCTCTGGCCTGGT
CCTGAGTCGTGTGCCCCATGTCCAGGGGGAACAGCAGGAGTGGGAGGGGACTGAGGAGCTGC
CGTCGCCTCCGGACCATGCCGAGAGGGCTGAAGAACAACATGAAAATACAGGCCCAGTCAG
GACCAGGGGCTCCCTGCTTCCCGGTGCTTGCGCTGCTGTGACCCCGGTACCTCCATGTACCC
GGCGACCGCCGTGCCCCAGATCAACATCACTATCTTGAAAGGGGAGAAGGGTGACCGCGGAG
ATCGAGGCCCTCAAGGGAAATATGGCAAAACAGGCTCAGCAGGGGCCAGGGGCCACACTGGA
CCCAAAGGGCAGAAGGGCTCCATGGGGGCCCCCTGGGGAGCGGTGCAAGAGCCACTACGCCGC
CTTTTCGGTGGGCGGGAAGAAGCCCATGCACAGCAACCACTACTACCAGACGGTGATCTTCG
ACACGGAGTTCGTGAACCTCTACGACCACTTCAACATGTTACCGGCAAGTTCTACTGCTAC
GTGCCCGGCTCTACTTCTTCAGCCTCAACGTGCACACCTGGAACCAGAAGGAGACCTACCT
GCACATCATGAAGAACGAGGAGGAGGTGGTGATCTTGTTTCGCGCAGGTGGGCGACCGCAGCA
TCATGCAAAGCCAGAGCCTGATGCTGGAGCTGCGAGAGCAGGACCAGGTGTGGGTACGCCTC
TACAAGGGCGAACGTGAGAACGCCATCTTCAGCGAGGAGCTGGACACCTACATCACCTTCAG
TGGCTACCTGGTCAAGCACGCCACCGAGCCCTAGCTGGCCGGCCACCTCCTTTCTCTCGCC
ACCTTCCACCCCTGCGCTGTGCTGACCCACCGCCTCTTCCCGATCCCTGGACTCCGACTC
CCTGGCTTTGGCATTCAGTGAGACGCCCTGCACACACAGAAAGCCAAAGCGATCGGTGCTCC
CAGATCCCGCAGCCTCTGGAGAGAGCTGACGGCAGATGAAATCACCAGGGCGGGGCACCCGC
GAGAACCCTCTGGGACCTTCCGCGGCCCTCTCTGCACACATCCTCAAGTGACCCCGCACGGC
GAGACGCGGGTGGCGGCAGGGCGTCCCAGGTGCGGCACCGCGGCTCCAGTCTTGGAATA
ATTAGGCAAATTCTAAAGGTCTCAAAGGAGCAAAGTAAACCGTGAGGACAAAGAAAAGGG
TTGTTATTTTTGTCTTTCCAGCCAGCCTGCTGGCTCCCAAGAGAGAGGCCCTTTTCAGTTGAG
ACTCTGCTTAAGAGAAGATCCAAAGTTAAAGCTCTGGGGTCAGGGGAGGGGCCCTTTCAGTTGAG
AAACTACCTCTGGCTTAATTCTTTTAAGCCACGTAGGAACCTTCTTGAGGGATAGGTGGACC
CTGACATCCCTGTGGCCTTGCCCAAGGGCTCTGCTGGTCTTTCTGAGTCACAGCTGCGAGGT
GATGGGGGCTGGGGCCCCAGGCGTCAGCCTCCCAGAGGGACAGCTGAGCCCCCTGCCTTGGC
TCCAGGTTGGTAGAAGCAGCCGAAGGGCTCCTGACAGTGGCCAGGGACCCCTGGGTCCCCCA
GGCCTGCAGATGTTTCTATGAGGGGCAGAGCTCCTTGGTACATCCATGTGTGGCTCTGCTCC
ACCCCTGTGCCACCCAGAGCCCTGGGGGGTGGTCTCCATGCCTGCCACCCTGGCATCGGCT
TTCTGTGCCGCTCCACACAAATCAGCCCCAGAAGGCCCGGGCCTTGGCTTCTGTTTTT
TATAAAACACCTCAAGCAGCACTGCAGTCTCCCATCTCCTCGTGGGCTAAGCATCACCGCTT
CCACGTGTGTGTGTGGTTGGCAGCAAGGCTGATCCAGACCCCTTCTGCCCCACTGCCCT
CATCCAGGCCTCTGACCAGTAGCCTGAGAGGGGCTTTTTCTAGGCTTCAGAGCAGGGGAGAG
CTGGAAGGGGCTAGAAAGCTCCCGCTTGCTGTGTTCTCAGGCTCCTGTGAGCCTCAGTCCTG
AGACCAGAGTCAAGAGGAAGTACACGTCCAATCACCCGTGTCAGGATTCACTCTCAGGAGC
TGGGTGGCAGGAGAGGCAATAGCCCTGTGGCAATTGCAGGACCAGCTGGAGCAGGGTTGCG
GTGTCTCCACGGTGCTCTCGCCCTGCCCATGGCCACCCAGACTCTGATCTCCAGGAACCCC
ATAGCCCTCTCCACCTCACCCATGTTGATGCCAGGGTCACTCTTGCTACCCGCTGGGCC
CCCAAACCCCGCTGCCTCTCTTCCCTTCCCCCATCCCCACCTGGTTTTGACTAATCCTGC
TTCCCTCTCTGGGCCTGGCTGCCGGGATCTGGGGTCCCTAAGTCCCTCTCTTTAAAGAACTT
CTGCGGGTCAGACTCTGAAGCCGAGTTGCTGTGGGCGTGCCCGGAAGCAGAGCGCCACACTC
GCTGCTTAAGCTCCCCAGCTCTTTCCAGAAAACATTAAACTCAGAATTGTGTTTTCAA

FIGURE 35

><subunit 1 of 1, 281 aa, 0 stop

><MW: 31743, pI: 6.83, NX(S/T): 1

><signal peptide>

MGSRGQGLLLAYCLLLAFASGLVLS

><start mature protein>

RVPHVQGEQQEWEGTEELPSPPDHAERAEEQHEKYRPSQDQGLPASRCLRCCDPGTSMP

ATAVPQI

><potential N-glycosylation site>

NITILK

><homology to ACR3_HUMAN 30 kd adipocyte complement-related
protein precursor from 99-end>

GEKGDGRGDRGLQGKYGKTGSAGARGHTGPKGQKGSMPGERCKSHYAAFSVGRKKPMHSNH

YYQTVIFDTEFVNLYDHFNMFTGKFYCYVPGLYFFSLNVHTWNQKETYLHIMKNEEEVVILF

AQVGDRSIMQSQSLMLELREQDQVWRLYKGERENAIFSEELDTYITFSGYLVKHATEP

FIGURE 36

GCGGAGCATCCGCTGCGGTCTCGCCGAGACCCCGCGCGGATTGCGCGGTCTTCCCGCGG
GCGCGACAGAGCTGTCCTCGCACCTGGATGGCAGCAGGGGCGCCGGGGTCCTCTCGACGCCA
GAGAGAAATCTCATCATCTGTGCAGCCTTCTTAAAGCAAAC TAAGACCAGAGGGAGGATTAT
CCTTGACCTTTGAAGACCAAACTAACTGAAATTTAAATGTTCTTCGGGGGAGAAGGGAG
CTTGACTTACACTTTGGTAATAATTTGCTTCCTGACACTAAGGCTGTCTGCTAGTCAGAATT
GCCTCAAAAAGAGTCTAGAAGATGTTGTCATTGACATCCAGTCATCTCTTTCTAAGGGAATC
AGAGGCAATGAGCCCGTATATACTTCAACTCAAGAAGACTGCATTAATTCTTGCTGTTCAAC
AAAAACATATCAGGGGACAAAGCATGTAAC TTGATGATCTTCGACACTCGAAAAACAGCTA
GACAACCCAACTGCTACCTATTTTTCTGTCCCAACGAGGAAGCCTGTCCATTGAAACCAGCA
AAAGGACTTATGAGTTACAGGATAATTACAGATTTTCCATCTTTGACCAGAAATTTGCCAAG
CCAAGAGTTACCCAGGAAGATTCTCTCTTACATGGCCAATTTTCACAAGCAGTCACTCCCC
TAGCCCATCATCACACAGATTATTCAAAGCCCACCGATATCTCATGGAGAGACACACTTTCT
CAGAAGTTTGGATCCTCAGATCACCTGGAGAACTATTTAAGATGGATGAAGCAAGTGCCCA
GCTCCTTGCTTATAAGGAAAAAGGCCATTCTCAGAGTTCACAATTTTCTCTGATCAAGAAA
TAGCTCATCTGCTGCCTGAAATGTGAGTGCCTCCAGCTACGGTGGCAGTTGCTTCTCCA
CATACCACCTCGGCTACTCCAAAGCCCGCCACCCTTCTACCCACCAATGCTTCAGTGACACC
TTCTGGGACTTCCCAGCCACAGCTGGCCACCACAGCTCCACCTGTAACCACTGTCACTTCTC
AGCCTCCCACGACCCTCATTTCTACAGTTTTTACACGGGCTGCGGCTACACTCCAAGCAATG
GCTACAACAGCAGTTCTGACTACCACCTTTCAGGCACCTACGGACTCGAAAGGCAGCTTAGA
AACCATAACCGTTTACAGAAATCTCCAAC TTAAC TTTGAACACAGGGAATGTGTATAACCC TA
CTGCAC TTTCTATGTCAAATGTGGAGTCTTCCACTATGAATAAACTGCTTCCTGGGAAGGT
AGGGAGGCCAGTCCAGGCAGTTCTTCCCAGGGCAGTGTTCCAGAAAATCAGTACGGCCTTCC
ATTTGAAAAATGGCTTCTTATCGGGTCCCTGCTCTTTGGTGTCTGTTCTCTGTTGATAGGCC
TCGTCTCTCTGGGTAGAATCCTTTCGGAATCACTCCGCAGGAAACGTTACTCAAGACTGGAT
TATTTGATCAATGGGATCTATGTGGACATCTAAGGATGGAAC TCGGTGTCTCTTAATTCATT
TAGTAACCAGAAGCCCAAATGCAATGAGTTTCTGCTGACTTGCTAGTCTTAGCAGGAGGTTG
TATTTTGAAGACAGGAAAATGCCCCCTTCTGCTTTCCTTTTTTTTTTTTGGAGACAGAGTCTT
GCTCTGTTGCCCAGGCTGGAGTGCAGTAGCACGATCTCGGCTCTCACCGCAACCTCCGTCTC
CTGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCTAAGTATCTGGGATTACAGGCATGTGCCA
CCACACCTGGGTGATTTTTGTATTTTAGTAGAGACGGGGTTTCACCATGTTGGTCAAGGCTG
GTCTCAAAC TCTGACCTAGTGATCCACCCTCTCGGCCTCCCAAAGTGCTGGGATTACAGG
CATGAGCCACCACAGCTGGCCCCCTTCTGTTTTATGTTTGGT TTTTGAAGGAATGAAGTG
GGAACCAAATTAGGTAATTTGGGTAACTGTCTCTAAAATATTAGCTAAAAACAAAGCTCT
ATGTAAAGTAATAAAGTATAATTGCCATATAAATTTCAAAT TCAACTGGCTTTTATGCAAA
GAAACAGGTTAGGACATCTAGGTCCAATTCATTCACATTCTTGGTTCAGATAAAATCAAC
TGTTTATATCAATTTCTAATGGATTTGCTTTTCTTTTATATGGATTCCTTTAAACTTATT
CCAGATGTAGTTCCTTCCAATTAAATATTTGAATAAATCTTTGTACTCAA

FIGURE 37

></usr/seqdb2/sst/DNA/Dnaseqs.min/ss.DNA45410

><subunit 1 of 1, 431 aa, 1 stop

><MW: 46810, pI: 6.45, NX(S/T): 6

MFFGGEGSLTYTLVIICFLTLRLSASQNCLKKSLEDVVIDIQSSLSKGIRGNEPVYTSTQED
CINCCSTKNISGDKACNLMIFDTRKTARQPNCYLFFCPNEEACPLKPAKGLMSYRIITDFP
SLTRNLPSQELPQEDSLHGGQFSQAVTPLAHHHTDYSKPTDISWRDTLSQKFGSSDHLEKLF
KMDEASAQLLAYKEKGHSQSSQFSSDQEIAHLLPENVSALPATVAVASPHTTSATPKPATLL
PTNASVTPSGTSQPQLATTAPPVTTVTSQPPTTLISTVFTRAAATLQAMATTAVLTTTFQAP
TDSKGSLETIPFTEISNLTGNTGVYNPTALSMNVESTMNKTASWEGREASPGSSSQGSV
PENQYGLPFKWLIGSLLFGVLFLVIGLVLLGRILSESLRRKYSRLDYLINGIYVDI

FIGURE 38

GCGGCACCTGGAAGATGCGCCCATTTGGCTGGTGGCCTGCTCAAGGTGGTGTTCGTGGTCTTC
GCCTCCTTGTGTGCCTGGTATTCGGGGTACCTGCTCGCAGAGCTCATTCCAGATGCACCCCT
GTCCAGTGCTGCCTATAGCATCCGCAGCATCGGGGAGAGGCCTGTCCTCAAAGCTCCAGTCC
CCAAAAGGCCAAAAATGTGACCACTGGACTCCCTGCCCATCTGACACCTATGCCTACAGGTTA
CTCAGCGGAGGTGGCAGAAGCAAGTACGCCAAAAATCTGCTTTGAGGATAACCTACTTATGGG
AGAACAGCTGGGAAATGTTGCCAGAGGAATAAACATTGCCATTGTCAACTATGTAAGTGGGA
ATGTGACAGCAACACGATGTTTTGATATGTATGAAGGCGATAACTCTGGACCGATGACAAAG
TTTATTCAGAGTGCTGCTCCAAAATCCCTGCTCTTCATGGTGACCTATGACGACGGAAGCAC
AAGACTGAATAACGATGCCAAGAATGCCATAGAAGCACTTGGGAAGTAAAGAAATCAGGAACA
TGAAATTCAGGTCTAGCTGGGTATTTATTGCAGCAAAAGGCTTGGAACTCCCTTCCGAAATT
CAGAGAGAAAAGATCAACCACTCTGATGCTAAGAACAACAGATATTCTGGCTGGCCTGCAGA
GATCCAGATAGAAGGCTGCATACCCAAAGAACGAAGCTGACACTGCAGGGTCCTGAGTAAAT
GTGTTCTGTATAAAACAAATGCAGCTGGAATCGCTCAAGAATCTTATTTTTCTAAATCCAACA
GCCCATATTTGATGAGTATTTTGGGTTTGTGTAAACCAATGAACATTTGCTAGTTGTATCA
AATCTTGGTACGCAGTATTTTATACCAGTATTTTATGTAGTGAAGATGTCAATTAGCAGGA
AACTAAAATGAATGGAAATTCCTTAAAAA

FIGURE 39

><signal peptide>

MRPLAGGLLKVVVFVVFASLC

><start mature protein>

AWYSGYLLAELIPDAPLSSAAYSIRSIGERPVLKAPVPKRQKCDHWTPCPSDTYAYRLLSGG

GRSKYAKICFEDNLLMGEQLGNVARGINIAIVNYVTG

><potential N-glycosylation site>

NVTATRCFDMYEGDNSGPMTKFIQSAAPKSLLFMVTYDDGSTRLNNDAKNAIEALGSKEIRN

MKFRSSWVFIAAKGLELPSEIQREKI

><potential N-glycosylation site>

NHSDAKNNRYSGWPAEIQIEGCIPKERS



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, 15/13, 15/62, 5/10, 1/21, 1/19, C07K 14/47, 16/18, C12Q 1/68		A3	(11) International Publication Number: WO 99/28462
(21) International Application Number: PCT/US98/25108		(43) International Publication Date: 10 June 1999 (10.06.99)	
(22) International Filing Date: 1 December 1998 (01.12.98)		YUAN, Jean [CN/US]; 176 West 37th Avenue, San Mateo, CA 94403 (US). BAKER, Kevin, P. [GB/US]; 1115 South Grant Street, San Mateo, CA 94402 (US). CHEN, Jian [CN/US]; 1860 Ogden Drive #4, Burlingame, CA 94010 (US).	
(30) Priority Data: 60/067,411 3 December 1997 (03.12.97) US 60/069,334 11 December 1997 (11.12.97) US 60/069,335 11 December 1997 (11.12.97) US 60/069,278 11 December 1997 (11.12.97) US 60/069,425 12 December 1997 (12.12.97) US 60/069,696 16 December 1997 (16.12.97) US 60/069,694 16 December 1997 (16.12.97) US 60/069,702 16 December 1997 (16.12.97) US 60/069,870 17 December 1997 (17.12.97) US 60/069,873 17 December 1997 (17.12.97) US 60/068,017 18 December 1997 (18.12.97) US 60/070,440 5 January 1998 (05.01.98) US 60/074,086 9 February 1998 (09.02.98) US 60/074,092 9 February 1998 (09.02.98) US 60/075,945 25 February 1998 (25.02.98) US		(74) Agents: DREGER, Walter, H. et al.; Flehr, Hohbach, Test, Albritton & Herbert LLP, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): GENENTECH, INC. [US/US]; Legal Dept. - MS49, One DNA Way, South San Francisco, CA 94080 (US).		Published With international search report.	
(72) Inventors; and (75) Inventors/Applicants (for US only): WOOD, William, I. [US/US]; 35 South Down Court, Hillsborough, CA 94010 (US). GODDARD, Audrey [CA/US]; 1920 Mason Street, San Francisco, CA 94113 (US). GURNEY, Austin, L. [US/US]; One Debbie Lane, Belmont, CA 94002 (US).		(88) Date of publication of the international search report: 14 October 1999 (14.10.99)	
(54) Title: POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME			
(57) Abstract			
<p>The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.</p>			

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INTERNATIONAL SEARCH REPORT

International Application No.

PC 98/25108

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/13 C12N15/62 C12N5/10 C12N1/21
 C12N1/19 C07K14/47 C07K16/18 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 99 06553 A (LACROIX BRUNO ;DUCLERT AYMERIC (FR); GENSET (FR); DUMAS MILNE EDWA) 11 February 1999 (1999-02-11) SEQ ID NO.147 claims 1-37</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-18

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

25 March 1999

Date of mailing of the international search report

21.07.99

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl;
 Fax: (+31-70) 340-3016

Authorized officer

HORNIG H.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/25108

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M.D. ADAMS ET AL.: "EST33760 Embryo, 12 week II Homo sapiens cDNA 5' end" EMBL SEQUENCE DATABASE, 18 April 1997 (1997-04-18), XP002097993 Heidelberg, FRG Accession no. AA330080; & M.D. ADAMS ET AL.: "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence" NATURE, vol. 377, 1995, pages 3-174, MACMILLAN JOURNALS LTD., LONDON,UK	1,5-10
X	--- T. FUJIWARA ET AL.: "Human aorta cDNA 5'-end Gen-308G12" EMBL SEQUENCE DATABASE, 29 August 1995 (1995-08-29), XP002097994 Heidelberg, FRG Accession no. D62632; ---	1,5-10
A	WO 97 07198 A (GENETICS INSTITUT) 27 February 1997 (1997-02-27) the whole document ---	1-18
A	WO 97 25427 A (GENETICS INST) 17 July 1997 (1997-07-17) the whole document ---	1-18
A	WO 96 14331 A (MERCK & CO INC ;STRADER CATHERINE D (US); CASCIERI MARGARET A (US)) 17 May 1996 (1996-05-17) the whole document ---	1-18
A	WO 91 02796 A (HSC RES DEV CORP ;UNIV MICHIGAN (US)) 7 March 1991 (1991-03-07) the whole document ---	1-18
A	IMAI T ET AL: "C33 ANTIGEN RECOGNIZED BY MONOCLONAL ANTIBODIES INHIBITORY TO HUMANT CELL LEUKEMIA VIRUS TYPE 1-INDUCED SYNCYTIIUM FORMATION IS A MEMBER OF A NEW FAMILY OF TRANSMEMBRANE PROTEINS INCLUDING CD9, CD37, CD53, AND CD63" JOURNAL OF IMMUNOLOGY, vol. 149, no. 9, 1 November 1992 (1992-11-01), pages 2879-2886, XP000610256 the whole document --- -/--	1-18

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAWASAKI E ET AL: "MOLECULAR CLONING AND CHARACTERIZATION OF THE HUMAN TRANSMEMBRANE PROTEIN TYROSINE PHOSPHATASE HOMOLOGUE, PHOGRIN, AN AUTOANTIGEN OF TYPE 1 DIABETES" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 227, no. 2, 14 October 1996 (1996-10-14), pages 440-447, XP002032722 the whole document	1-18
A	WO 95 30432 A (BOEHRINGER MANNHEIM GMBH ;MUELLER HANS WERNER (DE); JUNGHANS ULRIC) 16 November 1995 (1995-11-16) EMBL Sequence Database, Accession no. R87952; claims 1-5; figure 8	1-18
A	TASHIRO K ET AL: "SIGNAL SEQUENCE TRAP: A CLONING STRATEGY FOR SECRETED PROTEINS AND TYPE I MEMBRANE PROTEINS" SCIENCE, vol. 261, 30 July 1993 (1993-07-30), pages 600-603, XP000673204 the whole document	1-18
A	US 5 536 637 A (JACOBS KENNETH) 16 July 1996 (1996-07-16) cited in the application the whole document	1-18
A	KLEIN R D ET AL: "SELECTION FOR GENES ENCODING SECRETED PROTEINS AND RECEPTORS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, no. 14, 9 July 1996 (1996-07-09), pages 7108-7113, XP002061411 cited in the application the whole document	1-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/25108

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1-18, Partially.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: (1-18) partially

An isolated nucleic acid having at least 80% identity to a nucleotide sequence that encodes a PRO polypeptide consisting of the amino acid sequence of SEQ ID NO.2; said nucleotide sequence consisting of the sequence of SEQ ID NO.1; said nucleotide sequence comprising a nucleotide sequence consisting of the full-length coding sequence of SEQ ID NO.1; isolated nucleic acid which comprises the full-length coding sequence of the DNA deposited under accession no. ATCC 209526; a vector comprising said nucleic acid; a host cell comprising said vector; a process for producing a PRO polypeptide comprising culturing said host cell; isolated native sequence PRO polypeptide having at least 80% sequence identity to an amino acid sequence consisting of SEQ ID NO.2; isolated PRO polypeptide having at least 80% sequence identity to the amino acid sequence encoded by the nucleotide deposited under accession no. ATCC 209526; a chimeric molecule comprising said polypeptide fused to a heterologous amino acid sequence; an antibody which specifically binds to said PRO polypeptide;

2. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 6,7 and accession no. ATCC 209508;

3. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 14,15 and accession no. ATCC 209524;

4. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 18,19 and DNA28847 respectively DNA35877;

5. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 23,24,29,30 and accession no. ATCC 209528;

6. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 31,32 and accession no. ATCC 209530;

7. Claims: (1-18) partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject 1 but limited to SEQ ID NOs. 36,37 and
accession no. ATCC 209523;

8. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 41,42 and
accession no. ATCC 209492;

9. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 49,50 and
accession no. ATCC 209532;

10. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 54,55 and
accession no. ATCC 209531;

11. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 60,61 and
accession no. ATCC 209229;

12. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 68,69 and
accession no. ATCC 209527;

13. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 75,76 and
accession no. ATCC 209570;

14. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 85,86 and
accession no. ATCC 209618;

15. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 90,91 and
accession no. ATCC 209621;

16. Claims: (1-18) partially

Idem as subject 1 but limited to SEQ ID NOs. 98,99 and

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

accession no. ATCC 209619;

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/25108

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9906553	A	11-02-1999	AU 8555698 A	22-02-1999
WO 9707198	A	27-02-1997	US 5707829 A	13-01-1998
			AU 6712396 A	18-02-1997
			AU 6768596 A	12-03-1997
			CA 2227220 A	06-02-1997
			CA 2229208 A	27-02-1997
			EP 0839196 A	06-05-1998
			EP 0851875 A	08-07-1998
			WO 9704097 A	06-02-1997
WO 9725427	A	17-07-1997	AU 1532697 A	01-08-1997
WO 9614331	A	17-05-1996	EP 0791009 A	27-08-1997
			JP 10508486 T	25-08-1998
WO 9102796	A	07-03-1991	AU 647408 B	24-03-1994
			AU 6161690 A	03-04-1991
			CA 2066204 A	23-02-1991
			EP 0489058 A	10-06-1992
			JP 5500306 T	28-01-1993
			US 5585362 A	17-12-1996
			US 5434086 A	18-07-1995
			US 5543399 A	06-08-1996
			US 5776677 A	07-07-1998
			US 5240846 A	31-08-1993
			US 5863770 A	26-01-1999
WO 9530432	A	16-11-1995	AU 6926194 A	29-11-1995
			EP 0686397 A	13-12-1995
			JP 9503001 T	25-03-1997
US 5536637	A	16-07-1996	US 5712116 A	27-01-1998